

# **DS-11 Spectrophotometer/Fluorometer Series**

User Guide



Rev June 2016

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## 1. Introduction

### **Product Technology**

DeNovix Spectrophotometers / Fluorometers are compact instruments with an integrated processor and a high-resolution touchscreen. The software utilizes application specific icons along with the Android<sup>™</sup> operating system to provide an intuitive, easy-to-use instrument for both absorbance and fluorescence measurements.

The SmartPath<sup>®</sup> Technology automatically adjusts the microvolume mode pathlengths to enable a sample measurements across a broad concentration range. Absorbance range specifications can be found at www.denovix.com.

FX models include a fluorescence mode while the DS-11+ and DS-11 FX+ instruments also enable cuvette-based measurements for low concentration samples and basic absorbance kinetic studies.

#### Key Features include:

- Integrated Processor and Hi-Definition 7-inch Touchscreen
- Custom Android<sup>™</sup> Control Interface
- Simple, Intuitive EasyApps<sup>®</sup>
- 1 µL and Cuvette Full Spectrum UV-Vis Analysis
- Four Channel Fluorescence mode



## 2. Set up and Safety

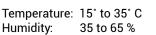
- 1. Remove all packing materials including the protective cover on the screen.
- 2. Plug the unit into a 100-240 VAC, 50-60/Hz receptacle using the grounded power supply (*CUI MPN: ETSA120330U-P5P-SZ, Transformer Dest Top 12V 40W, Lead free / RoHS Compliant*) included with each unit.
- 3. Turn on the power switch located in the back of the instrument. The Androidbased operating system will boot up in less than a minute.

#### Cautions

DO NOT REMOVE COVER

No operator serviceable components inside. Refer servicing to qualified personnel.

The DS-11 is designed for indoor use under the following conditions:



Use only the power supply provided with the instrument. Use of the instrument in any manner not specified by the manufacturer may impair the protection provided by the supplied power cord and power supply.

NE PAS OUVRIR L'APPAREIL. Aucun composant réparable par un utilisateur est inclus. Confiez l'entretien à du personnel qualifié . Le DS- 11 est conçu pour une utilisation en intérieur dans les conditions suivantes :

Température : 15° à 35°C Humidité: ......35 à 65%

Utilisez uniquement le bloc d'alimentation inclus avec l'instrument. Utiliser l'appareil dans des conditions non spécifiées par le fabricant peut compromettre la protection offerte par le cable d'alimentation et le bloc d'alimentation fournis avec l'appareil.





VCCI (Class B) compliance statement for users in Japan:

This is a Class B product based on the standard of the Voluntary Control Council for Interference (VCCI) from Information Technology Equipment. If this is used near a radio or television receiver in a domestic environment, it may cause radio interference. Install and use the equipment according to the instruction manual.

This equipment has been tested for radio frequency emissions and has been verified to meet CISPR 22.2 Class B.



この製品は、技術基準適合証明に関する条例等特定無 線設備(MPT省令第37号または1981)のタイプによっ て認定に準拠しています。この機器のカテゴリ:第2 条、第1項のアイテム(19)第2条第1項、項目(19)-2

#### **Instrument Rear Panel**

The DS-11 comes equipped with one Ethernet and three USB ports. The Ethernet feature is provided for internet connectivity only. The USB ports are used to facilitate data transfer to a FAT32 formatted USB storage device and connect to the accessory FX Module. In addition, the USB ports support the use of selected DYMO printers<sup>\*</sup> and an Opticon OPI 3601 USB barcode reader. USB mouse and keyboard devices may also be used with the DS-11 FX+ series of instruments.

\* Please refer to www.denovix for a list of supported Dymo printers.



## 3. Software Quick Guides

### Software Icons

+	Add		Select All	<	Export
	Delete	À	Purity Ratio Alert	<u> </u>	Sort
ATAN	Edit		Сору	Q,	Search
Ð	Undo		Paste	<b>(i)</b>	Info

- The **Overflow** (top action bar) is used to access secondary features such as Select Units, Screen Capture, User Guide and Exit.
- **Progress bars or busy indicators** are used to indicate the instrument is in the process of making a measurement. Please wait until the icon has disappeared before tapping the screen to perform any additional actions.
  - The **Quick Print** icon is only displayed on Report or Graph screens when a DYMO label printer is connected to the instrument.

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**Auto Export** status icons are displayed at the top right of a Run screen. Ensure WiFi is connected prior to making a measurement.

**Lock** icons are used in the absorbance Colorimetrics and Std Curve Methods apps to indicate whether a saved curve is in an editable state. Tap the icon change the status.

## **Software Buttons**

	The absorbance apps <b>Blank</b> button is used to establish a reference absorbance within each measurement app. The graph will not display a spectrum for a Blank measurement. The absorbance apps <b>Measure</b> button is used for sample measurements. It is inactive until at least one Blank measurement is complete.
	The fluorescence apps <b>Standards Measure</b> button is used to measure standards when building a standard curve.
	The <b>Samples Measure</b> button is used to quantitate samples.
·+ 0 +-	The fluorescence apps <b>Replicates On/Off</b> button is used to define if the standards curve enables one or up to three replicates.
	The fluorescence apps <b>Basic Measure</b> button is used to make single measurements not being read from a standard curve.
	Fields with <b>Drop-Down Menus</b> are indicated by black or white triangles located on the center bottom
	Fields with black underlines are editable by the user. Tapping on the field will enable a keyboard, a numeric keypad or a dialog box.
:	Application specific editor options can be accessed using the List Add/Edit button found to the right of some drop-down menus.
- <mark>- -</mark> -	<b>Options</b> buttons are used for such purposes as change units or trend line analysis parameters in the fluorescence apps.
$\checkmark$	An <b>accept and return</b> to previous screen button is found in the lower right of some screens. All changes made to a selection are immediate.



### **Software Navigation**

	Apps are launched using <b>Home</b> screen icons. Swipe the Home page to the left for additional icons.
alaa aa ara aa	The app <b>Run</b> , <b>Report</b> and <b>Graph</b> screens are accessed either by the tabs on the top bar or by swiping the screens left or right.
GENERAL ACCOUNT	Use the <b>Current Account Tab</b> drop-down menu on the top action bar in the Run, Report and Graph screens to select a specific account within an app. Use the <b>Accounts</b> app to create and manage accounts.
$\leftarrow$	The bottom bar <b>Back</b> button is used to navigate to the main level of the current app.
$\Box$	The bottom bar <b>Home</b> button is used to navigate to the Home screen without closing the app.
	The bottom bar <b>Recents</b> button is used to as a means of displaying recently used apps. Swipe the app to the left from the Recents list will close the app.
$\sim$	The bottom bar <b>Collapse</b> button is used in place of the Back button whenever a keyboard is enabled. Use this button to dismiss the keyboard.

## **Notification Bar**

Information such as available updates, scheduled system backups reminders and WiFi connectivity status are located on the bottom action bar adjacent to the date and time display. Swipe the notification up for details. Tap outside the notification box to minimize the information.

## **Top Bar Overflow Functions**

Screen Capture	A .jpg may be exported as described in the chapter entitled "Screen Capture and Export" using this option.
Export Selected Samples	A .csv file of sample data may be may be exported as described in the chapter entitled "Screen Capture and Export". Network printing is also enabled using this option.
Change Units	Concentration units may be changed in some apps using this option.
Archive Selected Samples	The <b>Archive</b> function exports data as a .csv file to a network folder or USB flash drive and <b>permanently</b> deletes it from the instrument.
Clear Samples	This function saves and then clears all current sample records from the Report and Graph screens.
Baseline Correction	Some apps enable <b>Baseline Correction</b> changes from the Overflow function on the Run screen.
User Guide	The <b>User Guide</b> may be accessed either from the Overflow icon within any app or by launching the User Guide app.
About	The <b>About</b> feature brings up an information box displaying the current app version number.
Lamp Reset	The Diagnostics app <b>Lamp Reset</b> is used as a means to re- optimize a lamp when necessary.
Tech Support	The Diagnostics app <b>Tech Support</b> feature is used to send instrument related diagnostics files to DeNovix customer support.
Exit	Use the <b>Exit</b> function to close an application.



## 4. Report and Graph Screens

The measurement apps generally use a three page structure consisting of the Run, Report and Graph screens. The various screens are accessed either by using the top tabs or by swiping the touch screen left or right.

The typical Run screen varies significantly between the absorbance and the fluorescence apps. Refer to the "Basic Operation" section for the absorbance mode and the individual fluorescent app sections for more information.

#### **Report Screen**

d and a second	sDNA run	REPORT	GRAPH					GE	NERAL ACCOUNT
	s 🔊	Î							<
#	Sample Name	Conc.	Units	Factor	A260	mm	260/280	260/230	Date Time
	BLANK								2016-03-03 09:57
1	example A	376.87	ng/µL	50.00	7.537	10	1.84	2.17	2016-03-03 09:57
2	example B	376.70	ng/µL	50.00	7.534	10	1.85	2.18	2016-03-03 09:58
3	example C	375.52	ng/µL	50.00	7.510	10	1.84	2.19	2016-03-03 09:58

	¥	ð	Mar 3, 9:59 AM ᄛ
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- The Report screen list includes application specific data fields for each sample measured in the current measurement session.
- Tap rows to highlight and select data for export. The **Select All** /**UnSelect All** icons are used to quickly select or deselect all rows.
- The change will be applied to the current Report and Graph screen rows as well as to the sample information retrieved using the Data app.



- Clear highlighted sample records from the both the Report and Graph screens using the **Delete** icon. The data is removed from the current report but is still available using the Data app.
- Highlighted sample data may be exported or printed using the top action bar Overflow **Screen Capture** or **Export Selected** functions. Additional information is provided in the chapter entitled "Screen Capture and Export".

#### **Graph Screen**



- Tap rows to highlight and select data to display the spectra. The Select All / UnSelect All icons are used to quickly select or deselect all rows.
- Up to 24 samples may be overlaid on the graph. Absorbance apps will display sample spectra while the fluorescence apps will display the concentration calculated from the standard curve trend line.
- Color blocks are used as a legend convention.



- Highlight a single row and then tap the **Edit** icon to add or modify a sample name. The change will be applied to the current Report and Graph screen rows as well as to the sample information retrieved using the Data app.
- Clear highlighted sample records from the both the Report and Graph screens using the **Delete** icon. The cleared data will be available using the Data app.
- Highlighted sample data may be exported or printed using the top action bar Overflow **Screen Capture** or **Export Selected** functions. The data will will exported as a .csv file and spectra may be re-graphed using a spreadsheet chart function. Additional information is provided in the chapter entitled "Screen Capture and Export".



## 5. Absorbance Apps: Basic Operation

#### **Microvolume Measurements**

- 1. Ensure both top and bottom sample surfaces are clean.
- 2. Pipette 1 µL of the blank solution onto the lower sample surface. Lower the top arm and tap the **Blank** button.
- 3. Remove the solution from both sample surfaces using a clean, dry lab wipe.
- 4. Pipette 1 µL of the sample solution onto the lower sample surface.
- 5. Lower the arm and tap the **Measure** button. All microvolume measurements are reported in 10 mm equivalent absorbance values.

#### **Cuvette Measurements**

Refer to the light path arrow as a guide when inserting a cuvette. Use cuvettes that meet the following specifications:

- Width: 12.5 mm Length: 12.5 mm Height: 45 mm Z height: 8.5 mm
- UV wavelength region: Use quartz or UV transparent plastic cuvettes
- Visible wavelength region: Use quartz or plastic cuvettes
- 1. Insert a cuvette pre-filled with the Blank solution. Lower the arm and tap the **Blank** button.
- 2. Insert a cuvette pre-filled with the sample solution. Lower the arm and tap the **Measure** button.



#### **Auto Run Measurements**

- 1. Perform a Blank measurement as previously described and then select the **Auto Run** checkbox on the app Run screen. Pipette 1  $\mu$ L of the sample solution onto the lower sample surface.
- 2. Lower the arm for automatic measurements. Deselect the feature to perform new Blank measurements.

Note: This feature may be used for both microvolume and cuvette mode measurements.

#### Sample Measurement Modes

- Measurement mode selections are made using the drop-down menu found next to the Measure button on the Run screen.
- The **Microvolume** mode uses 1.0 µL samples pipetted onto the sample surface.
- The microvolume **Short Path** mode requires 0.5 µL samples pipetted onto the sample surface. This mode is for high concentration samples and does not use the 0.5 mm pathlength.
- The microvolume **Fast Mode** option is primarily for samples in the range measured by the 0.5 mm pathlength although it may be used for higher concentration samples .
- The DS-11+ enables the use of 10, 5, 2 and 1 mm pathlength Cuvette modes.
  - Report screens will include actual absorbance values measured using a specific cuvette pathlength. Graph screens will display all cuvette absorbance values normalized to a 10 mm value.
- A new **Blank** measurement must be made when changing between microvolume and cuvette modes.





## **Typical Absorbance Run Screen**

- Blank, Measure, AutoRun and Mode: Use the Blank button when measuring the reference solution and the Measure button for samples. Tap the AutoRun box to enable automatic measurements upon lowering the arm. Use the drop-down menu to select either microvolume or cuvette mode measurements.
- Sample Name: Tap the input box to display an on-screen keyboard or use a Opticon OPI 3601 USB scanner to enter sample names.
- Sample Type and Factor Fields: Displays current sample type and associated factors used for concentration calculations. The fields may be fixed or selectable depending on the application.
- Absorbance Graph: The spectral plot displays data for the current sample normalized to a 10 mm pathlength for microvolume measurements. Pinch and zoom anywhere in the graph to rescale the X or Y-axis.
- Baseline Correction nm: Displayed at bottom of screen. Use the top action bar **Overflow** function to modify. Additional baseline correction details are provided within each relevant measurement app chapter.



#### **Screen Saver**

- The backlight for the screen will automatically turn off after 10 minutes of inactivity. Simply tap anywhere on the screen to resume the last function.
- Although the screen is turned off for the screen saver mode, functions such as the Timer and Kinetics measurements continue in the background. Always check that these apps are not in use in the background prior to turning off the instrument.

#### **Mechanism Busy Indicator**

- A busy indicator will be displayed on the screen when the measurement mechanism is busy.
- It is normal for the busy indicator to stop and start during mechanism operations.
- Please do not lift the arm, push software buttons or turn the instrument off while a busy indicator is displayed.

#### **Measurement Sessions**

- New measurement sessions begin each time an app is accessed from a closed state or when the account is changed while the application is open.
- In addition, new sessions automatically begin when the number of sample records on the Report screen reaches 9999.



#### **Best Practices Absorbance Measurements**

- Clean both sample measurement surfaces prior to making the Blank measurement.
- Use dH<sub>2</sub>O, not detergents or alcohol, for routine sample measurement surface cleaning.
- Use a dry lab wipe to remove measurement liquids from both the top and bottom surfaces immediately after each measurement.
- Use 1 µL samples for routine microvolume measurements.
   Note: Select the Short Path mode for high concentration 0.5 µL samples.
- Use a fresh aliquot for each measurement.
- Use a fresh tip to deliver each sample aliquot.
- Avoid introducing bubbles when pipetting samples onto the measurement surfaces.
- Ensure sample concentrations fall within absorbance limits of the instrument for accurate results.
- Use the same buffer a sample is suspended in for the Blank measurements.
- Avoid using buffers such as RIPA buffers that contain components with strong absorbances at the wavelength of interest.
- Measure a fresh aliquot of the buffer using the Measure button and ensure the spectrum is relatively flat along the baseline before measuring samples.
- For cuvette mode measurements, use cuvettes with 8.5 mm Z heights. Select the Cuvette option using the drop-down menu next to the Measure button and ensure the cuvette is inserted in the proper orientation.



## 6. Absorbance Nucleic Acid Apps



The four preconfigured nucleic acid apps are used to quantify nucleic acid samples based upon absorbance values at 260 nm.

### **Quick Protocol**

- 1. Launch the appropriate application from the Home screen.
- 2. Establish a Blank using the appropriate buffer.
- 3. Enter a sample name.
- 4. Select a sample type if applicable.
  - Enter optional user-defined ng-cm/µL factor (ssDNA and Microarray).
  - Select a dye type (Microarray only).
- 5. Measure a fresh aliquot of sample using the **Measure** button.

#### Notes:

- Blank with TE if the sample is dissolved in TE.
- Ensure that the sample isolation procedure is optimized and that samples are purified prior to making absorbance measurements.
- Ensure all solutions are homogenous and well-mixed prior to sampling.
- Use fresh aliquots for each microvolume measurement.



### dsDNA, RNA and ssDNA

The basic nucleic acid apps are optimized for the factor used to calculate sample concentrations.

#### **Screen Features**

- Sample Type: The sample type is used to determine which ng-cm/µL factor is used to calculate the concentration.
  - The sample type is fixed for both the dsDNA and RNA apps.
  - A **Factor Input** option along with the default ssDNA sample type is available from the selector field for the ssDNA app.
- Factor. The default wavelength-dependent factors used for each app are as follows: dsDNA: 50 ng-cm/µL RNA: 40 ng-cm/µL ssDNA: 33 ng-cm/µL
  - Oligo factors derived from the Calculator app may be copied and pasted directly into this field.
- Concentration Results: Concentration results are reported in units of ng/µL.
  - Units may be changed using the Run screen top action bar Overflow menu. The units selected at the time of the measurement is what is saved to the database.
- A260: Reports the baseline-corrected 260 nm absorbance value.
- 260/230: Displays the ratio of the 260 nm and 230 nm absorbance values.
- 260/280: Displays the ratio of the 260 nm and 280 nm absorbance values.

Note: The DNA and RNA apps do not distinguish between the nucleic acid species within a sample. It is important to optimize the isolation protocol to ensure accurate quantitation.



#### Microarray

The Microarray app is used to quantify nucleic acid samples based upon absorbance values at 260 nm and dyes based upon dye specific analysis wavelengths.

#### **Screen Features**

- Sample Type: The sample type selection of ssDNA is the default option for this application. Options include:
  - ssDNA (Factor = 33 for nucleic acid concentration calculation.)
  - RNA (Factor = 40 for nucleic acid concentration calculation.)
  - Factor Input to enable a user-defined nucleic acid factor. Oligo factors derived from the Calculator app may be copied and pasted directly into this field.
- Dye Type: Cy3 is default selection for this application.
- Nucleic Acid Concentration Results: Results are reported in units of ng/µL.
  - Units may be changed using the Run screen top action bar Overflow menu. The units selected at the time of the measurement is what is saved to the database.
- Dye/Fluorophore Concentration Results: Results are reported in units of µM.
- A260\*: Reports the baseline and dye corrected 260 nm absorbance value. This value may be lower than the A260 value observed in the absorbance spectra due to the correction factors applied.
- AXXX: The baseline-corrected dye specific analysis wavelength absorbance value (e.g A550 when using the Cy3 dye).



## **Dye List**

There are 11 preconfigured dye types available for both the Microarray and Labeled Proteins applications. New dye types may be saved using the List Add/ Edit button to the right of the dye list drop-down menu. Refer to the manufacturer of the dye for analysis nm, extinction coefficient and correction factor information.

Dye	Ext. Coeff	A260 Factor	A280 Factor
СуЗ	150000	0.08	0.08
Cy5	250000	0.05	0.05
Cy5.5	250000	0.05	0.18
Alexa Fluor 405	34,000	0.23	0.7
Alexa Fluor 488	71,000	0.3	0.11
Alexa Fluor 555	150,000	0.08	0.08
Alexa Fluor 594	90,000	0.43	0.56
Alexa Fluor 647	239,000	0	0.03
Alexa Fluor 680	184,000	0	0.05
Alexa Fluor 700	192,000	0	0.07
Fluorescein/FITC	68000	0.32	0.2

Dyes saved to the list are accessible for both the Microarray and Labeled Proteins apps. The new dyes may be used by all accounts, including the General Account. The user-added dyes may only be edited or deleted by the account holder or an administrator account holder.



The following information is required in order to save a new dye to the list:

- Dye name
- · Analysis wavelength
- Analysis wavelength specific molar absorption coefficient (M<sup>-1</sup> cm<sup>-1</sup>)
- 260 nm absorbance correction factor
- 280 nm absorbance correction factor

Preconfigured dyes may not be modified or deleted.

#### **Baseline Correction for Dyes**

The software anchors the visual spectrum display to the baseline at 750 nm for all measurements. It automatically applies a 340 nm bichromatic normalization for the A260 value and uses a sloping baseline between 400 and 750 nm for dye concentration calculations.

If adding a new dye, it is important to empirically determine if the default 750 nm selection is appropriate to use as the baseline correction wavelength.

- The baseline correction screen is accessed from the Run screen top action bar Overflow icon.
- Tap the Add icon to include a user-defined option between 750 and 840 nm.
- The new option will be used for both the the visual display and the sloping dye baseline.
- Once a baseline correction nm is selected, it is applied to all subsequent measurements and is not dye specific. When changing dyes, always confirm that the proper baseline correction is applied.



## **Nucleic Acid Concentration Calculations**

A modified Beer-Lambert equation is used to calculate concentrations as follows:

c = (A \* e)/b

- c = the nucleic acid concentration in ng/microliter
- A = the absorbance in AU
- e = the wavelength-dependent factor in ng-cm/microliter
- b= the pathlength in cm (10 mm)

#### **Baseline Correction**

Baseline corrections correct for offsets due to sample particulates. If a baseline correction is not used, the sample spectrum may be offset from the baseline, resulting in a change in the reported sample concentration. The DS-11 uses an optimized baseline value as the default selection. However, the user may elect to define the baseline nm used for specific measurements.

The baseline correction screen is accessed from the Run screen top action bar Overflow icon. Once a baseline correction nm is selected, it is applied to all subsequent measurements within the same measurement session.

### **Purity Ratios**

- The generally accepted 260/280 values are ~1.8 for pure DNA and ~2.0 for RNA.
- The 260/230 values for nucleic acids usually range between 1.8 and 2.2.



## **Troubleshooting Purity Ratios**

lssue	Probable Causes/ Solutions
Low 260/230	Dirty measurement surfaces or improper Blank. Clean the surfaces, measure a new Blank using fresh source of dH <sub>2</sub> 0 or sample buffer. Use the same solution (water or buffer) the sample is in for the blank measurement.
	Presence of residual extraction reagent (ex. Carbohydrates, Chaotropic salts, phenol).
	Re-purify the sample, then remeasure. Contact the manufacturer of the extraction kit for guidance on how to optimize the procedure.
	Dirty measurement surfaces or improper Blank.
High 260/230	Clean the surfaces, measure a new Blank using fresh source of $dH_20$ or sample buffer. Use the same solution (water or buffer) the sample is in for the blank measurement.
	Dirty measurement surfaces or improper Blank.
	Clean the surfaces, measure a new Blank using fresh source of dH <sub>2</sub> 0 or sample buffer. Use the same solution (water or buffer) the sample is in for the blank measurement.
Low 260/280	
	Presence of residual extraction reagent (ex. protein, phenol). Re-purify the sample, then remeasure. Contact the manufacturer of the extraction kit for guidance on how to optimize the procedure.
High 260/230	Dirty measurement surfaces or improper Blank. Clean the surfaces, measure a new Blank using fresh source of dH <sub>2</sub> 0 or sample buffer. Use the same solution (water or buffer) the sample is in for the blank measurement.



#### **Ratio Alerts**

The DS-11 software v3.0 and above displays alerts icons within the dsDNA, RNA and ssDNA apps for samples with purity ratios outside of specified minimum and maximum limits. Tapping on the icon will pop-up a message with error specific information.

The default settings are as follows:

Ratio	Minimum	Maximum
260/230	1.8	3.0
260/280	1.65	2.5

Users may elect not to have the alerts show or set custom limits using the Run screen Overflow menu. The new limits may be used by all accounts, including the General Account. Saved user-added limits may only be edited or deleted by the account holder or an administrator account holder.

Note: The limits are only enforced for samples above a minimum concentration value. A blue warning icon will be displayed when the sample concentration is below the threshold.



## 7. Absorbance Purified Protein, Peptide Apps



### **Quick Protocol**

- 1. Launch the appropriate application from the Home screen.
- 2. Establish a **Blank** using the appropriate buffer.
- 3. Enter a sample name.
- 4. Select a sample type.
  - New protein or peptide types may be added to the selection list.
  - Select a dye type when using the Labeled Proteins app.
- 5. Measure a fresh aliquot of sample using the **Measure** button.

#### Notes:

If the buffer exhibits significant absorbance at 280 nm, use alternative methods such as colorimetric assays to quantitate proteins.
Ensure all solutions are homogenous and well-mixed prior to sampling.
Use fresh aliguots for each microvolume measurement.



#### Protein A280

The Protein A280 application is used to quantify samples based upon absorbance values at 280 nm.

#### **Screen Features**

• Sample Type: The sample type and associated mass extinction coefficients options include:

Sample Type	E1% at 280 nm for 10 mm pathlength	
BSA	6.67	
1A=1mg/mL	10	
lgG	13.7	
E1%	User-defined <b>mass</b> extinction coefficient (L gm <sup>-1</sup> cm <sup>-1</sup> ) for a 10 mg/mL (1%) solution	
MW & Ext Coeff	User-defined MW (Daltons) and <b>molar</b> extinction coefficient $(M^{-1} \text{ cm}^{-1})$	

Use the 1A=1mg/ml option when neither the E1% nor Ext Coeff and MW for the purified protein sample are known.

- · Concentration Results: Results are reported in units of mg/mL.
  - Units may be changed using the Run screen top action bar Overflow menu. The units selected at the time of the measurement is what is saved to the database.
- A280: Reports the baseline-corrected 280 nm absorbance value.
- 260/280: Displays the ratio of the 260 and 280 nm absorbance values.



### **Protein Type List**

New Protein types using either user-defined E1% or MW and Ext. Coeff values may be saved using the List Add/Edit button to the right of the sample type drop-down menu.

- The new sample types may be used by any account holder.
- The new sample types may be edited or deleted only by the account holder or an administrator account.

#### **Baseline Correction**

Bichromatic normalizations correct for baseline offsets due to sample particulates. If a baseline correction is not used, the sample spectrum may be offset from the baseline, resulting in a change in the reported sample concentration. The DS-11 uses an optimized baseline value as the default selection. However, the user may elect to define the baseline nm used for specific measurements.

The baseline correction screen is accessed from the Run screen top action bar Overflow icon. Once a baseline correction nm is selected, it is applied to all subsequent measurements within the same measurement session.



### **Labeled Proteins**

The Labeled Protein application is used to quantify protein samples based upon absorbance values at 280 nm as well as Dyes and Fluorophores at specific analysis wavelengths.

#### **Screen Features**

• Sample Type: The sample type and associated mass extinction coefficients options include:

Sample Type	E1% at 280 nm for 10 mm pathlength	
BSA	6.67	
1A=1mg/mL	10	
lgG	13.7	
E1%	User-defined <b>mass</b> extinction coefficient (L gm <sup>-1</sup> cm <sup>-1</sup> ) for a 10 mg/mL (1%) solution	
MW & Ext Coeff	User-defined MW (Daltons) and <b>molar</b> extinction coefficient $(M^{-1} \text{ cm}^{-1})$	

- Dye Type: Cy3 is default selection for this application.
- Protein Concentration Results: Results are reported in units of mg/mL.
  - Units may be changed using the Run screen top action bar Overflow menu. The units selected at the time of the measurement is what is saved to the database.
- Dye/Fluorophore Concentration Results: Results are reported in units of µM.
- A280\*: Reports the baseline and dye corrected 280 nm absorbance value. This value may be lower than the A280 value observed in the absorbance spectra due to the correction factors applied.
- AXXX: The baseline-corrected dye specific analysis wavelength absorbance value (e.g A550 when using the Cy3 dye).



### **Protein Type List**

New Protein types using either user-defined E1% or MW and Ext. Coeff values may be saved using the List Add/Edit button to the right of the sample type drop-down menu.

- The new sample types may be used by any account holder.
- The new sample types may be edited or deleted only by the account holder or an administrator account.

#### **Dye List**

There are 11 preconfigured dye types available for both the Microarray and Labeled Proteins applications. Refer to the "Microarray" section of the chapter entitled "Nucleic Acids" for more information.

#### **Baseline Correction**

The software anchors the visual spectrum display to the baseline at 750 nm for all measurements. It automatically applies a 340 nm bichromatic normalization for the A280 value and uses a sloping baseline between 400 and 750 nm for dye concentration calculations.

If adding a new dye, it is important to empirically determine if the default 750 nm selection is appropriate to use as the baseline correction wavelength.

- The baseline correction screen is accessed from the Overflow icon.
- Tap the Add icon to include a user-defined option between 750 and 840 nm.
- The new option will be used for both the the visual display and the sloping dye baseline.
- Once a baseline correction nm is selected, it is applied to all subsequent measurements and is not dye specific. When changing dyes, always confirm that the proper baseline correction is applied.



### Peptides

The Peptide app is used for low concentration samples which do not have aromatic residues such as Trp, Tyr or Cys-Cys disulphide bonds. The Protein A280 app is recommended when the sample does contain aromatic rings.

#### **Screen Features**

- Sample Type: The first two sample types included in the sample type dropdown are preconfigured for both the analysis wavelength and the corresponding E 0.1%.
- Sample type and associated E 0.1% coefficients options include:

Sample Type	E 0.1% at specified nm for 10 mm pathlength	Analysis Wavelength
e215 nm	11.7	215 nm (recommended)
e205 nm	31	205 nm
E0.1%	User-defined <b>mass</b> extinction coefficient (L gm <sup>-1</sup> cm <sup>-1</sup> ) for a 1 mg/mL (0.1%) solution	215 or 205 nm User Option

- Analysis Wavelength Selector. The radio button selector will appear for all sample types except the preconfigured e215 nm and e205 nm options. Note: Extinction coefficients are wavelength dependent.
- Concentration Results: Results are reported in units of mg/mL.
  - Units may be changed using the Run screen Overflow menu. The units selected at the time of the measurement is what is saved to the database.
- AXXX: Reports the baseline-corrected analysis wavelength absorbance values (e.g A205 or A215).



#### **Protein and Peptide Concentration Calculations**

The Beer-Lambert equation is used to calculate concentrations as follows:

A = E1% \* b \* c

c = the protein concentration in g/100 mL A = the absorbance in 10 mm equivalent E1%= the percent extinction coefficient (L gm<sup>-1</sup> cm<sup>-1</sup>) for a 10 mg/mL (1%) solution b= the pathlength in cm (10 mm)

The following equation is used to convert g/100 mL to mg/mL concentrations:

c (mg/mL)= (A/E1%) \*10

The relationship between percent extinction coefficient (E1%) and molar extinction coefficient (Ext. Coeff) is:

E1% = (Ext. Coeff \*10) / molecular weight of protein (Daltons)

Note: The Peptide app uses E 0.1% values whereas the Protein A280 app uses E 1% values.

The DeNovix software reports concentrations in mg/mL units for the Protein A280, Labeled Protein and Peptide applications. The concentration is determined based upon the absorbance value at the analysis wavelength used for the measurement.



#### **Ratio Alerts**

The DS-11 software v3.0 and above displays alerts icons within the Protein A280 for samples with A260/280 purity ratios outside of specified maximum limits. Tapping on the icon will pop-up a message with error specific information.

In general, pure protein samples will have a ratio below 0.57. The default software value that will trigger an alert is 0.7.

Users may elect not to have the alerts show or set custom limits using the Run screen Overflow menu. The new limits may be used by all accounts, including the General Account. Saved user-added limits may only be edited or deleted by the account holder or an administrator account holder.

Note: The limit is only enforced for samples above a minimum concentration value. A blue warning icon will be displayed when the sample concentration is below the threshold.



## 8. Absorbance Colorimetrics App



Refer to the manufacturer of the assay reagent for assay specific instructions.

### **Quick Protocol**

- 1. Launch the app from the **Home** screen.
- 2. Select the assay type on Run screen. Navigate to the Standard Curve screen.
- 3. Select the appropriate microvolume or cuvette mode prior to making the Blank measurement.
- 4. Establish a **Blank** using the appropriate buffer or the 0.00 mg/mL standard.
- 5. Generate a new Standard Curve by measuring at least one replicate for a minimum of 2 standard concentrations. Alternatively, apply a previously saved curve selected from the drop-down menu.
- 6. Navigate to the **Run** screen. Enter a sample name.
  - Optional: Enter a dilution factor to be applied to the reported concentration.
- 7. Quantitate the sample using the Measure button.Notes:
  - The Graph Flip button is available on the Run and Graph Screens in the Colorimetrics and Custom Standard Curve apps. The flip option is not a available when reviewing the results in the Data app. Only the absorbance style graphs will be displayed.
  - Standard Concentrations: Minimum 2, Maximum 8.



## **Generating a Standard Curve**

- 1. Use the drop-down menu on the Std Curve screen to select **New Curve** or to import a saved standard curve or standard curve concentration list.
- If the New Curve option is selected, tap the Enter Standards button to enable a pop-up box to enter a list of standard concentrations for the current measurement session.
  - The table enables up to 7 standard concentrations in addition to the predefined 0.00.
  - The 0.00 concentration is used for the diluent (no analyte) plus the assay reagent. Although not recommended, the 0.00 concentration may be edited.
- Measure at least one replicate of each standard concentration by highlighting the standard concentration row and then tapping the Measure button.
  - Up to 5 replicates may be measured for each concentration. Use fresh aliquots for each measurement when using the microvolume mode.
  - The software will advance after each measurement until the fifth replicate has been measured. If additional measurements are made, the fifth position will be overwritten.
  - A minimum of two standards must be measured before the **Measure** button is active on the Run screen.
  - Select a standard row or individual replicate then tap the **Delete** icon to display a drop-down list of delete options.
  - Use the Undo icon to restore the most recent edit or delete action.



# Line Fit

The average of the replicates is used to determine a best-fit trend line of the data set. The trend line equation is applied to the sample absorbance values to calculate concentrations.

Options include:

- Linear
- 2<sup>nd</sup> Order
- Interpolation: This option draws a straight line between each point.

## Saving a Standard Curve

A standard curve or a series of standard concentrations may be saved and reused at a later date.

200	Colorim	etrics RUN	STD CURVE	REPI	ORT C	GRAPH			GENERAL ACCOUNT
l	]				Saved C Values or (		w Curve		Linear
	Blank	Measure	Microvolume		BCA	. (	Enter Sto	Is	Save Conc. Values
2.5					mg/mL	Abs 1	Abs 2	Abs 3	View Conc. Values list
			Q. Q. (	2	0.000	ADS I	ADS Z	ADS 3	Save Std Curve
2									View Std Curve list
0 1.5	·								
10 mm Absorbance									
≘ 0.5									
0									
	0 0.2	0.4 Concentration	0.6 0.8	ì					
÷							¥	ه ا	1ar 3, 10:12 AM ᄛ

• Use the List Add/Edit button to the right of the curve list drop-down menu to save a curve or concentration list.



# **Editing a Standard Curve**

- The curve is in an editable state when the Lock icon is green.
- When a previously saved curve or concentration list is imported, a red Lock icon will appear. Tap the icon to unlock the curve data set to enable modifications and new absorbance measurements.
- All modifications to a curve (new or saved) are effective immediately. It is
  important to save a curve with a new name prior to making any changes if
  you do not want to modify the original curve.
- The only way to recall absorbance data for a standard curve is to save the results as a saved curve before exiting the app.
- Although all accounts may use any previously saved standard curve or saved concentration list, only the curve account holder or an administrator may modify or delete a saved curve or concentration list.

### **Exporting a Standard Curve**

The standard curve replicate values may be exported using the Screen Capture function described in the chapter entitled "Screen Capture and Export."

Note: The standard curve data is not available in a .csv format. Use the Screen Capture function to export a .jpg showing the curve replicate values.



# BCA

The bicinchoninic acid assay (BCA assay) is used to quantify protein by measuring the Cu-BCA chelate formed in the presence of protein.

• The reaction product is measured at 562 nm using a baseline correction at 750 nm.

# Bradford

The Bradford assay is based upon a protein-induced absorbance shift of a Coomassie Blue dye as a method to determine protein concentration.

• The reaction product is measured at 595 nm using a baseline correction at 750 nm.

Note: Some Bradford formulations may form particulates during prolonged incubations. It is recommended that the cuvette option be used when using these types of formulations. The particulates may interfere with the shorter pathlengths used for the microvolume measurements.

### Lowry

The modified Lowry assay is used to quantify proteins based upon the absorbance of tetradentate copper-protein complexes.

• The reaction product is measured at 650 nm using a baseline correction at 405 nm.

# Pierce 660

The Pierce 660 nm Protein Assay is used to measure a reddish-brown dyemetal complex that changes to green upon protein binding.

• The reaction product is measured at 660 nm using a baseline correction at 750 nm.



# 9. Absorbance UV-Vis App



The UV-Vis app is used to monitor up to 6 specific analysis wavelengths. Absorbance values for all wavelengths from the user selected wavelength ranges are saved for each measurement and can be exported as a .csv file using the Export function from the Report or Graph screens.

# **Quick Protocol**

- 1. Launch the UV-Vis application from the Home screen.
- 2. Establish a **Blank** using the appropriate buffer.
- 3. Enter the analysis nm in the first input field. This wavelength is used to determine which pathlength is utilized by the SmartPath® technology for microvolume measurements. Enter up to five additional wavelengths of interest.
- 4. Enter a sample name.
- 5. Measure a fresh aliquot of sample using the **Measure** button.

# **Wavelength List**

A list of between 1 to 6 wavelengths may be saved and recalled by using the Overflow icon to the right of the wavelength list drop-down menu.



### **Wavelength Range Selection**

There are two wavelength ranges available for the UV-Vis app. Use the checkbox feature to select between the 220 to 750 nm or the 190 to 840 nm options. Changing ranges will automatically save and then clear current results. A new Blank is required when switching ranges.

Use the Custom Formula App to create a method that utilizes a user-defined wavelength range.

### **Cursor Position**

The UV-Vis app Run Screen graph includes an moveable cursor initially set at the analysis wavelength. Moving the cursor allows the user to find the absorbance value of any wavelength after a measurement is made.

Note: The cursor wavelength absorbance value is a temporary value not saved by the software.

### **Baseline Correction**

The baseline correction normalizes the entire spectrum using the absorbance value of the wavelength specified and corrects for baseline offsets due to sample particulates. The default selection is set to 750 nm for the UV-Vis app.

If a baseline correction is not used, the sample spectrum may be offset from the baseline, resulting in a change in the reported sample concentration.

The baseline correction list is accessed from the Overflow icon. Once a baseline correction nm is selected, it is applied to all subsequent measurements within the same measurement session.



# 10. Absorbance OD 600 App



This application is generally used to determine the optical density of microbial cell cultures at 600 nm. Conversions to cells per mL are based upon a user-defined factor.

## **Quick Protocol**

- 1. Launch the app from the **Home** screen.
- 2. Establish a **Blank** using the appropriate buffer.
- 3. Enter a sample name.
- 4. Enter a cell conversion factor (optional).
- 5. Measure a fresh aliquot of sample using the Measure button.

Notes:

•All cell type specific conversion factors are user-derived.

•Ensure culture is well mixed prior to sampling.

•Use fresh 1 uL aliquots for each microvolume measurement.

•Although microvolume OD 600 measurements can be made, the cuvette 10 mm mode is recommended for this app. This option is available on the DS-11 FX +.



### **Unique Features**

- A600: The OD 600 is a measure of the light scattered by the cell suspension solution. Reported values may differ for the same sample measured on different spectrophotometer systems as values are dependent on both the cell type and the optical configuration of the spectrophotometer.
- It is recommended that an optimal harvest density be empirically determined for each microbial cell type when using a new spectrophotometer.
- **Cell Number Conversion Factor**: The cell number conversion factor is used to convert the 600 nm value into the number of cells per mL for the sample by multiplying the absorbance value by the factor. Cell numbers are reported in terms of 1 \* 10<sup>8</sup> cells. The factor must be entered into the appropriate field prior to a measurement.

Note: The cuvette mode selection (10 mm, 5 mm, 2 mm or 1 mm) must be taken into account when determining target OD 600 values or entering cell conversion factors. When making microvolume measurements, the OD 600 values reported and used for cell number calculations are 10 mm equivalent values.

#### **Baseline Correction**

As microbial cell culture OD 600 values are measurements of light scattering, the DS-11 software does not use a bichromatic baseline normalization unless specified as a user selection.

The baseline correction screen is accessed from the Overflow icon. Once a baseline correction nm is selected, it is applied to all subsequent measurements within the same measurement session.



# 11. Absorbance Kinetics App



**Kinetics** 

The Kinetics application is a cuvette mode only application available for the DS-11+ and DS-11 FX+ models.

## Quick Protocol

- 1. Launch the app from the **Home** screen.
- 2. Use the **Create Method** button on the **Run** screen to define the parameters of the method.
  - The button will change to a drop-down menu after at least one method has been defined. Use the List Add/Edit button to the right of the dropdown to define additional new methods or edit previously saved methods.
- 3. Navigate to the **Run** screen and tap the **Heater** button to preheat the cuvette block to the selected temperature.
- 4. When the Heater temperature field displays the set temperature, insert the cuvette and establish a **Blank** using the appropriate buffer.
- Insert the cuvette with the sample solution and tap the **Measure** button. 5.

#### Notes:

•Use cuvettes with Z heights of 8.5 mm. Use the light path arrow as a guide when inserting a cuvette into the cuvette block.

•Use a quartz or UV transparent plastic cuvette when using methods that include the UV wavelength range.



## **Define Method Screen Features**

- Method Name: A unique name is required for each method.
- Analysis nm: Enter the primary wavelength of interest for the method. The absorbance value at this wavelength will be plotted versus time on the Trend plot.
- Analysis nm 2: Optional 2nd wavelength of interest for the method. The absorbance value at this wavelength will be plotted versus time on the Trend plot.
- Wavelength Range: Define a range between 190 to 840 nm.
- **Baseline Correction Wavelength**: 750 nm is the general recommendation but the optimal wavelength should be empirically determined.
- Heater Set Point: The heater may be set to temperatures between 37°C to 45°C.

### **Stage Selections**

Up to three stages of timed intervals may be included for each method. The parameters for each stage include:

- Delay: This is the time before the first measurement of each stage.
- Interval: This is the time between measurements. The minimum interval is 5 seconds.
- Duration: This is the cumulative time of the individual stage **not** including the delay. A duration will be rounded down to to the nearest even multiple of the interval.

Example: Interval = 10 seconds, Duration = 31 seconds Duration rounded down to 30 seconds.



### **Run Screen Features**

- Method: Select the method of choice using the drop-down menu. Use the List Add/Edit button to the right of the drop-down to create or edit methods. All methods are available to all accounts. However, only the account holder or an administrator may edit or delete a method.
- Graph Type Flip Button: Graph types may be switched during the run.
  - Spectra: Absorbance vs wavelength
  - Trend: Absorbance vs elapsed time for analysis wavelength(s)
- Heater Control: Tap the On/Off button to control the heater. The heater may overshoot the method set point before reaching a equilibrium ± 0.5°C of the set point.
- Timer. This feature is used to monitor the sample solution warm-up time. The timer is used in a count-up only mode but is not used to track the measurement time.
- Stop Button: A Stop button replaces the Measure button on the Run screen during the Kinetics run. Tapping this button will end the measurement and save all data accumulated up to that point. The Measure button will be available when the run is finished.
- **Results Table**: A scrollable table with the absorbance values at the specified wavelength(s) per time is displayed on the Run screen.

#### **Heater Temperature**

- The temperature display box is color-coded for quick reference:
  - White: Heater is off.
  - Yellow: Heater is on, heater is not at method set point.
  - Orange: Heater is on, cuvette holder block has reached the method set point within <u>+</u> 0.5°C.



### Sample Solution Temperature

• The solution in the cuvette may not reach the set point as quickly as the heater. Use the Run screen timer as a means of monitoring the elapsed warm-up time. Below are estimated warm-up times.

Solution Start Temp		4ºC	Room Temperature		
Heater Set Point Temp	37ºC	45°C	37ºC	45°C	
Warm-up Time (mins)	15 to 20	15 to 20	10 to15	10 to 15	

The times were determined after a 10 mm plastic disposable cuvette filled with water was inserted into the cuvette block already heated to the method set point. Actual times may vary and should be empirically determined for each assay protocol.

• The temperature display box does not measure the temperature of the solution in the cuvette.

### **Report, Graph, Trend Screens**

• **Report Screen**: Unlike other DS-11 measurement apps, the results displayed in the Kinetics Report screen list are associated with one assay run. Each time a new assay run is initiated, the previous results will be saved and then cleared from the Report, Graph and Trend screens. The cleared data may be accessed using the Data app.

Note: It is normal for the software to sometimes experience minor delays in reporting kinetics data information relative to the actual measurement time point. The actual time each measurement is initiated is what is reported in the Elapsed Time field.

- **Graph Screen**: Displays the absorbance for the method-defined wavelength range for each time point.
- Trend Screen: Displays the absorbance vs. elapsed time for method-defined analysis wavelength(s). Use the action bar timers to narrow the time points for export or screen capture.



# 12. Absorbance Custom Methods Apps



These applications are used to create, save and access user-defined methods.

## **Quick Protocol**

- 1. Launch the applicable Custom Methods app from the Home screen.
- 2. Use the **Create Method** button on the **Run** screen to define the parameters of the method.
  - The button will change to a drop-down menu after at least one method has been defined.
  - Use the List Add/Edit button to the right of the drop-down to define additional new methods or edit previously saved methods.
  - All methods are available to all accounts. However, only the account holder or an administrator may edit or delete a method.
- 3. Establish a **Blank** using the appropriate buffer. If the method is a standard curve type, establish the Blank and measure the standards from the **Std Curve** screen before navigating to the **Run** screen.
- 4. Enter a sample name.
- 5. Measure a fresh aliquot of sample using the **Measure** button.



# **Primary Settings**

Each new method requires a unique name. In addition, the following parameters must be defined:

- Analysis nm: Enter the primary wavelength of interest for the method.
  - Absorbance values at the analysis nm will be used to generate a curve for Standard Curve methods. The analysis nm will be also be used to determine which path length is utilized by the SmartPath® technology for microvolume measurements.
- Baseline nm: A baseline correction wavelength is required for the Standard Curve methods but not for the Formula Methods. Bichromatic normalizations correct for baseline offsets due to sample particulates. If a baseline correction is not used, the sample spectrum may be offset from the baseline.
  - Although the general recommendation would be to use 340 nm for UV only wavelength ranges and 750 nm for full range methods, the optimal baseline correction wavelength should be empirically determined.
- Wavelength Range: The software will perform a wavelength optimization function each time the wavelength range is defined or edited within a method.

# **Custom Standard Curve Methods**

In addition to the primary settings described above, the Standard Curve app also requires that a units label be defined.

- Once a new method is established or a previously saved method is selected, navigate to the Std Curves screen and follow the steps outlined for Generating a Standard Curve in the chapter entitled "Colorimterics".
- Standard Curve methods function the same way as preconfigured Colorimetrics apps.



# **Custom Formula Methods**

In addition to the primary analysis wavelength, the Formula Methods app enables the user to include up to 4 additional results for each measurement. The result fields may be used to monitor additional specific wavelengths or have the software perform routine calculations based upon absorbance values measured at specific wavelengths.

- Result Name: Enter a name for each custom formula.
- **Custom Formulas**: Tap on the custom formulas input field to display a popup dialog. The following functions are available:

Function	Description		
Numerical Operations	+ add - subtract * multiply ÷ divide		
A(x)	Absorbance at wavelength x		
Log(x)	Log of x		
ln(x)	Natural Log of x		
٨	<b>Exponent</b> Example 4^3 equals 4x4X4		
$\checkmark$	Square Root		
e	<b>Scientific notation</b> Example 2e3 equals 2 x 10 <sup>3</sup> or 2000		
V1, V2 Variables that are entered on the Run s prior to each measurement.			

 Advanced: When checked, this feature allows the user to designate that the method uses a Savitzky–Golay filter instead of the default boxcar method to smooth data.



### **Formula Guidelines**

• Use the A(x) button on the input screen to use the absorbance value result of a specific wavelength in the formula. Enter the wavelength within the parenthesis.

Example: A(260)

• Check that closed sets of parenthesis are included when entering complex formulas.

Example: Log(A(260)) is correct. Log(A(260) is incomplete.

• Do not enter an equal sign before the equation. This is implied within the software.

Example: A(260)/A(280) is correct while = A(260)/A(280) is incorrect.

- The software automatically applies the baseline correction specified in the method set-up. Do not include baseline corrections in user-defined equations.
- 10 mm pathlength equivalent absorbance values are used and reported for microvolume measurements.



# 13. Fluorescence: Basic Operation

The DeNovix spectrophotometer/fluorometer software versions 2.0 and above include both absorbance and fluorescence apps. The fluorescence app icons will appear on the first page of the Home screen for the integrated DS-11 FX and DS-11 FX+ models. The inactive versions of the icons will appear on the second page for DS11 or DS-11+ units when a FX Module USB accessory is not detected.

LEDs	Excitation Filter Range	Emission Filter Range
UV	361-389 nm	435-485 nm
Blue	442-497 nm	514-567 nm
Green	490558 nm	565-650 nm
Red	613-662 nm	664-740 nm

The table below lists the four LEDs included in all FX models.

# **Fluorescence Measurements**

- 1. If using the FX Module accessory, connect the module to the DS-11/DS-11+ using one of the three back USB ports.
- 2. Insert a micro tube pre-filled with the fluorescent solution. Close the cover and tap **Measure**.

#### Notes:

- Use clear thin-walled 0.5 mL plastic PCR tubes only
- Measure 200 µl volume samples.



### **Best Practices**

- Ensure all sample concentrations in the thin walled assay tubes fall within the limits of the assay or reagent kit for accurate results.
- Use 200 µL volumes in a clear thin walled PCR tube.
- Lower the cover before tapping the measure button.
- Avoid introducing air bubbles into the sample solution when mixing samples.
- Follow assay reagent manufacturers' recommendations regarding temperature, incubation time, and protection from ambient light.
- Follow assay reagent manufacturers' recommendations regarding suggested standard curve concentrations and data analysis.
- Ensure all samples and standards are treated identically in terms of incubation times and temperature.
- Minimize assay tube and solution temperature fluctuations as these may impact the accuracy of the assay. Samples at higher temperatures will generally have lower fluorescence.
- Do not label the side of an assay tube as this could interfere with the sample measurement.



# 14. Fluorescence: Standard Curve Method Apps

The five apps listed below are designed using the same basic architecture. Each app (except the Custom Std Curves app) includes preconfigured assays designed to accommodate commonly used commercially-available reagent kits.

	Fluoro dsDNA	<ul> <li>•DeNovix dsDNA Broad Range</li> <li>•DeNovix dsDNA High Sensitivity</li> <li>•DeNovix dsDNA Ultra High Sensitivity</li> <li>•Quant-iT<sup>™</sup> PicoGreen</li> <li>•Quant-iT<sup>™</sup> dsDNA HS</li> <li>•Quant-iT<sup>™</sup> dsDNA BR</li> </ul>	•Qubit™ dsDNA BR •Qubit™ dsDNA HS •Quantifluor dsDNA •Quantifluor One dsDNA
	Fluoro RNA	•Quant-iT™ RiboGreen •Quant-iT™ RNA HS •Quant-iT™ RNA BR •Qubit™ RNA HS	•Qubit™ RNA BR •Quantifluor RNA •Quant-iT™ microRNA •Qubit™ microRNA
	Fluoro ssDNA	•Quant-iT™ OliGreen •Qubit™ ssDNA •Quantifluor ssDNA	
	Fluoro Protein	•Quant-iT™ Protein •Qubit™ Protein	
05000 00000	Fluoro Std Assays	•All entries to be defined by the user	



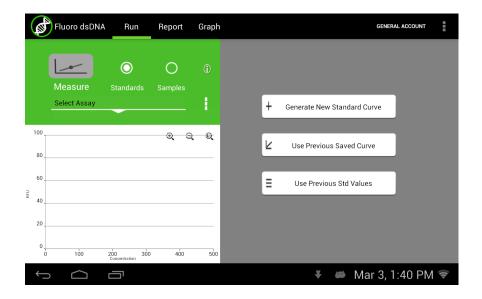
# **Quick Protocol**

- 1. Launch the app from the **Home** screen.
- 2. Select the assay type using the drop-down menu on the **Run** screen.
- 3. Generate a new curve:
  - Alternatively, import a saved curve or list of sample concentrations
- 4. Insert the standard (200 uL in a thin walled PCR tube) into the sample holder and lower the cover. Measure at least one replicate for a minimum of 2 standard concentrations.
- 5. Tap the Samples radio button. Enter a sample name.
  - Optional: Enter a dilution factor to be applied to the reported concentration.
- 6. Quantitate a sample using the **Measure** button.

#### Refer to the manufacturer of the assay reagent for assay specific instructions.

#### Note:

•DeNovix and Qubit assays will have predefined standard concentrations and specified units.



# **Run Screen- Assay and Curve Selections**

#### **Assay Selection**

- Select an assay from the drop-down menu in the left panel.
- Preconfigured entries are optimized in regards to excitation source, assay units, and trend line analysis according to the reagent kit manufacturer's recommendations.
- New assays may be entered using the List Add/Edit button.
- User defined assays may be used by all accounts but may be modified or deleted only by the account owner.



### **Curve Options**

#### **Generate New Curve**

- Choosing this option will pop-up a dialog window used to enter the concentration values for the standards.
- The standard concentrations for preconfigured Qubit<sup>®</sup> assays are hardcoded into the software. The standard concentrations for all other assays are determined by the user at the time of the assay.
- Although trend line analysis options and concentration units used to generate the standard curve are preset for preconfigured assays, they may be changed using the advanced options button.
  - **Blank:** If selected, the first row in the standards table is used to measure a Blank. The average RFU value of the blank measurements will be subtracted from all standard and sample measurements. If the Blank is not selected, the initial concentration for preconfigured assays will be set to 0.00. This standard is typically the reagent/fluorophore solution without any nucleic acid or protein.
  - **Trend Line:** If selected, the trend line will be forced through the lowest standard concentration. When a Blank is used, the trend line will be forced through the graph origin (0,0).

#### Use Previous Curve or Std Values

- Choosing this option will pop-up a dialog window with a radio button selector for saved standard curves and saved standard curve concentration lists.
- All modifications to a curve or list are effective immediately. It is important to save the selection with a new name prior to making any changes if you do not want to modify the original curve or standards list. Changes to a curve do not affect previously measured samples.
- Although all saved lists and saved curves are available for use by all accounts, only the account holder may modify the selection.

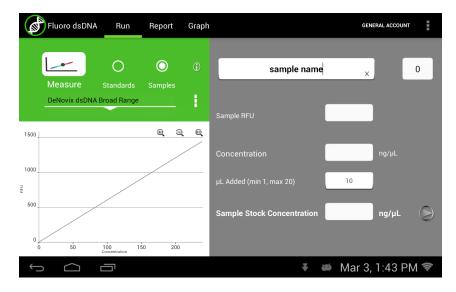


# **Run Screen- Standard Curve View**

	Fluoro dsDNA	Run	Report	Graph	1	GENERAL ACCOUNT
		$\bigcirc$	$\bigcirc$	Ĵ		Linear
	Measure		Samples		BLANK	t 🕆 🕆
	DeNovix dsDNA E	Broad Range		1	200.0	
100			- Q. Q.	Q		
80						Save As
60						+ New Curve
<sup>∏</sup> 40.						
20						Replicates
0	b 50	100 15 Concentration	io 200			Last Modified 16-03-03 01:41
<u> </u>					¥	🛎 Mar 3, 1:42 PM 📚

- The **Stds** button will pop-up the Edit Concentrations for Standard Curve window.
- The **Delete and Undo** icons are used to manage the RFU values within the table.
- The **Save** button will pop-up the Save Values window. Either concentration values or concentration values with the associated RFU values may be saved.
- The **New Curve** button is used to generate a new curve or load a previously saved curve. All sample data generated using the Samples radio button will be automatically saved and accessible using the Data app.
- The Replicates arrow button enables the use of up to three replicate measurements per standard concentration. The average of the replicates will be used to generate the trend line.





# **Run Screen- Sample Results View**

- Use the **Samples** radio button to display the measurement results view.
- The samples **Measure** button will be disabled until one replicate of at least two standards are measured using the standard curve view.
- The **Concentration from Curve** results are calculated directly from the standard curve trend line. Results are reported in terms of the units used to derive the trend line.
- The **Optional Dilution Factor** (or the **µl Added** value for Qubit<sup>™</sup> assays) will be applied as a multiplier to the Concentration from Curve value and the result reported in the Sample Stock Concentration field.
- The Sample Stock Concentration results are reported in terms of the units selected by the user. The value saved in Report will be displayed in terms of the units in use at the time of the measurement.



### New Standard Curve Assays/Methods

Each new method requires a unique name. In addition, the following parameters must be defined:

#### **Excitation Source**

- Use LED Radio buttons to select the excitation source.
- The **LED Wizard** may be used to determine the optimal excitation source if not known. The software will make a measurement using each of the three excitation sources and will report the associated RFU in the color-coded boxes.
- Some fluorophores do not exhibit significant fluorescence unless bound to a protein or nucleic acid. It may be necessary to set up an assay reaction to best use the LED Wizard.
- The standard curve assay methods use one excitation source to determine the concentration of a sample based upon the RFU measured using the corresponding emission filter set.

#### **Advanced Options**

• Use the **Advanced Option** buttons to set both the Trend Line/Blank selections as well as the standard curve units.

Although all accounts may use any saved method, only the method account holder or an administrator may modify or delete the new method.



# 15. Basic Fluorometer App

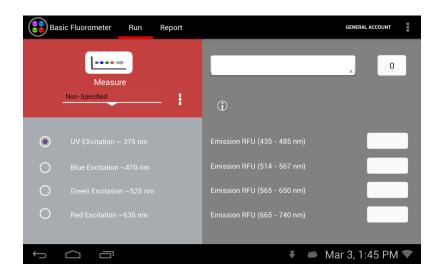


This app enables the DS-11 FX series of instruments to be used as a versatile fluorometer for applications such as dye QC checks and assay development. The app is especially useful for fluorophores with significant Stoke shifts as it allows the user to excite a sample with one LED and get RFU results from multiple emission channels.

# **Quick Protocol**

- 1. Launch the app from the **Home** screen.
- 2. Select the Fluorophore type using the drop-down menu on the **Run** screen. Alternatively, select an excitation LED using the radio buttons.
- 3. Insert the standard (200  $\mu$ L in a thin walled PCR tube) into the FX mode sample holder.
- 4. Optional: Enter a sample name.
- 5. Tap the **Measure** button.





### **Excitation Selection and Emission Results**

- Select a fluorophore from the drop-down menu in the left panel. The excitation source is automatically determined for preconfigured fluorophores.
- The selected LED is the **only** excitation source used during a measurement. However, the emission RFU will be collected for each longer wavelength emission filter set.
  - For example, if the blue LED is selected, the the emission results for the blue, green and red channels will be reported. If the green LED is selected, only the green and red channel results will be reported.
- New fluorophores may be entered using the List Add/Edit button.
  - The Add button will pop-up a window that includes a LED Wizard. Tapping the Wizard button will result in the sample being excited by each LED and the RFU measured by the corresponding detector. The LED that resulted in the largest RFU at its detector will automatically be chosen as the LED for the method



# 16. Data Export and Print Options

### **Export Options**

The data associated with highlighted samples on a Report or Graph screen may be exported via email, sent to a network folder or saved to a FAT32 formatted USB flash drive as .csv files.

- Data may be automatically exported to a network folder after every measurement. Refer to the "Network Folder Set-up" section for more information.
- To open an exported .csv file on a computer, the DeNovix instrument and the computer must **both** be set to use either period or comma number formats. The DS-11 FX number format is determined by the language selected using the Settings app.

### **Export Formats**

#### **Standard Formats**

• The default .csv file format for each absorbance measurement app includes all absorbance vs wavelength data for the app specific measurement wavelength range as well as the calculated data displayed on the app Report screen.

#### **Custom Formats**

- User specific formats may be defined using the **Export Formats** option accessed from the Report screen Overflow menu.
- The format used when exporting results is selected at the time of the export. Accounts other than the General Account may set a user-defined format as the default selection using the Accounts app.



#### **Defining a New Format:**

- Provide a title for the format.
- Hold the arrow icon and drag a column up or down to change the order of the columns.
- Touch on the column name and swipe it left or right to exclude or include it in the exported report.
- Tap on the input field to change the name of a column header in the exported report.

### **Graph Screen Exports**

- Selected sample results exported from an absorbance app Graph screen follow the same format as when exported for the Report screen. Graphs are not exported as part of the .csv file.
- Use the Screen Capture option to export the current graph via email or to save the .jpg image to a network folder or a FAT32 formatted USB flash.

# **Export Via Email**

- Screen captures as well as data .csv files may be exported via email.
- Use the Account app Overflow Address Book function to save frequently used recipient email addresses.
- It is recommended that a new, dedicated POP3 **Gmail** account be used for all outgoing email from the DS-11 (except for instruments used in China). This type of account works very well with the instrument's operating system and prevents personal incoming email from being stored on the unit.



# Email Set-up

- 1. Ensure that the instrument is connected to WiFi or Ethernet.
- 2. Swipe the Home screen to the left to navigate to the second page, launch the Email app and follow the prompts using the Manual setup option.
- 3. Input Incoming Server Information:
  - Select the POP3 option on the Account Types screen. Do not use an IMAP account as messages from IMAP accounts could occupy a significant amount of the instrument's memory.
- 4. Set the inbox checking frequency to "Never" on the Account Options screen. The DS-11 email should be used only to export data and screen captures.
- 5. Input Outgoing Server Information:
  - · Use settings appropriate for the lab email server.
- 6. Enter a name to display on outgoing messages.

Refer to your local IT support group if additional assistance is required.

### **Export to a Network Folder**

- Screen captures, data report .csv files, as well as instrument backups and data archive files may be sent to a selected network folder.
- The two supported transfer options include Server Message Block (SMB) and File Transfer Protocol (FTP).
- Use the following apps to manage network folder options on the DS-11 FX:



#### Settings App

• Permission to setup a network folder for the General Account as well as standard user accounts are enabled or disabled by an administrator using the Permissions option in the Settings app. "Enabled" is the default selection.

#### Accounts App

• Network folder paths are defined and saved on a per user account basis from the Accounts app. The primary Administrator network folders are used only for the Backup and Restore function in the Settings app.

### **Network Set-up**

- 1. Contact your local IT group to confirm that you have access to a local server or FTP site. Ensure that the instrument is connected to WiFi or Ethernet.
- 2. Launch the Accounts app and select a user account.
- 3. Use the top drop-down menu in the right pane to select the **Add New Network Folder** function.
- 4. Use the List Add/Edit button to add a network folder to the drop-down menu.
- 5. Select either the SMB or FTP option.
- 6. Enter a **nickname** for the network path. This is the name displayed in all drop-down menus referencing the network folder.
- 7. Enter the server computer's **host name**. Contact your local IT group for additional information.



- 8. Select log-in option. Contact your local IT group to request log-in credentials.
  - Log in: Enter the network folder username and password required to access the selected network folder.
  - Anonymous: No user name or password required.
  - File (SMB only): Upload a configuration file (file extension .prp) from a USB device. Note: If a username or password is entered that is different from information contained in the config file, the manually typed information will be used in validating the path access.
  - Refer to <u>http://jcifs.samba.org/src/docs/api/overview-summary.html#scp</u> for additional guidance.
- 9. Use the Browse button to locate and select a specific folder path.
- 10. Use the **Auto Report** checkbox to enable automatic export of sample data after every measurement.

### USB

- Screen captures as well as data .csv files may be saved to a FAT32 formatted USB flash drive.
- There are three USB ports on the back of the instrument although only one storage device is recognized at a time. The two additional ports may be used for the FX Module accessory as well as for selected DYMO printers and barcode readers.



## **Auto Export Icon**

When the auto export feature is selected, a status icon will appear at the top right of the screen after the first measurement.



- Automatic export of every sample measurement to a network folder is enabled on a per user account basis.
- The auto export feature may only be selected for one saved network folder per user account.
- Automatic exports will be stored in a single file per app type per user account per day.
- When selected, the auto export will apply to all measurement apps used by the applicable account.

Note: All sample data is saved to the instrument even if automatic exports fail due to server connectivity issues.

### **Troubleshooting Tips**

- Confirm that there is WiFi or Ethernet connectivity. Use the Browser app on the second page of the Home screen to confirm connectivity or check the connectivity status icon on the right of the bottom navigation bar.
- Confirm that the log in credentials are valid for the network folder option selected and that the hostname is correct.
- For internet connectivity issues, network folder access failures and all other server related issues, contact your local IT support group.



# **Print Options**

The DS-11 FX software supports printing to networked printers or to selected DYMO printers\*. All Dymo print-outs are in black and white.

\*Please refer to <u>www.denovix</u> for a list of supported Dymo printers.

#### **WiFi and Networked Printers**

Screen shots as well as selected sample data and graphs may be printed to both Wifi enabled and networked printers.

- Use the Overflow menu to select either Screen Capture or Export Selected Samples to bring up a dialog box with Network Printer as a radio button option.
- Selecting this option will display a preview of the print-out along with many customizable options.
- To select a printer, ensure that the instrument has WiFi or Ethernet connection and then tap the first item listed under Printer Options. This will bring up a list of available printers. If no printers are listed, tap the Network Printers icon in the left pane to search for nearby printers.

The current software supports approximately 90% of printers on the market. DeNovix will continue to add new drivers to accommodate as many new printers as possible in future software updates but cannot guarantee compatibility with all printers.

Please contact DeNovix or your local distributor for a list of recommended printers if your current printer is not compatible with the software.



### **DYMO Printers**

- The software enables the use of three DYMO paper or label sizes:
  - 4x6 labels (DYMO 4XL only) and 2.5 inch paper rolls are used to print screen captures and selected sample results from the Report or Graph screens.
    - Report and Graph print column formats will be as shown on the instrument display.
  - 1 x 0.5 inch labels are used to print sample names, sample concentrations, units and the date on individual labels suitable for standard microfuge tubes.
    - When used with the Colorimterics or Custom Standard Curves app, the concentration printed is the dilution factor applied recalculated value.
- A Quick Print button will be displayed on the Run, Report and Graph screens when a DYMO printer is detected. Label sizes may be selected by tapping the printer icon displayed next to the date and time in the bottom notification bar.
- Although sample names may include up to 48 characters, the DYMO printed label will print 2 rows of characters per sample name. The exact number of sample name characters may vary depending on the language in use.



# **17. Utility Apps and Functions**



### **Accounts App**

User accounts manage default settings within specific apps as well as saved sample and dye types, wavelength lists and user methods. Accounts are also used as a means of segregating data when performing searches in the Data app.

The **Add New Network Folder** function is accessed from a drop-down menu on the right side of the screen. The **Address Book** function accessed from the Overflow menu in this app allows users to save frequently used recipient email addresses for easy reference.

#### System Accounts

There are two preconfigured system accounts built into the software:

- Primary Administrator
  - This account is used to manage instrument settings. It is not used within the measurement applications.
  - The primary administrator account is not initially password protected. A password may be applied at any time.
- General Account
  - This account is used as the default selection for all apps each time an app is opened. This account may not be password protected or deleted.



#### **User Added Accounts**

All added administrator or standard user accounts may be password protected.

- Administrator Accounts
  - An administrator level account may only be created by another administrator.
  - Administrator accounts may modify or delete user entries of any sort created from within any account.
  - Administrators may change the password for any account as well as delete any account.
- Standard Accounts
  - Any user may add a standard level account.
  - Standard accounts may only modify or delete sample or dye types, wavelength lists or user methods created from within their account.

If an account is deleted, all associated sample and dye types, wavelength lists and user methods are still available for general use unless deleted by an administrator.

Contact the holder of an administrator account if a standard user account password is forgotten. Contact DeNovix customer support or your local distributor if the Primary Administrator password is forgotten. Please have the serial number of the instrument available when requesting help for the Primary Administrator password reset.

## **App Default Selections**

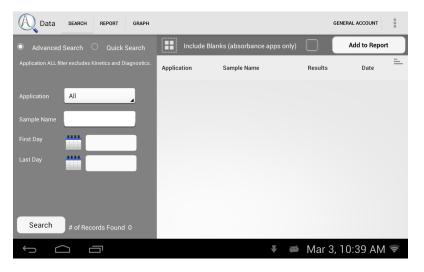
Some application specific default settings may be defined on a per account basis. Launch the Accounts app from the Home screen and then use the drop-down menus to select the account, the app and the setting of interest.

Note: The default selections for the General Account may not be modified.



#### **Data App**

This app allows the user to search for past data using either a Advanced Search or Quick Search option. Data can then be added to the Report and Graph screens for review and export. In addition, data may be archived from the Search Screen.



#### **Search Options**

- Data is automatically filtered based upon the Account selected using the tab on the top action bar.
- The **Advanced Search** allows multiple filters to be applied in a single search. The **Quick Search** option enables the user to filter by a single criteria in addition to the automatic Account filter.
- Kinetics and Diagnostics results are not accessible using Quick Search.
- Once the filter criteria have been selected, tap the **Search** button at the bottom of the screen. Tap any column header to sort the results list.



- Highlight the sample(s) of interest and tap the **Add to Report** button to include the samples on the Report and Graph screens. Blank measurements may be included in the list but will not provide any absorbance data.
- All data must be from the same app type for the samples to be added to the Report or Graph screens.
  - Note: The Data app does not include a Graph screen for fluorescence apps.

# **Export vs Archive**

- All data is automatically saved to the DS-11 FX onboard computer database at the time of measurement.
  - The Auto Export feature is available when a network folder is set-up using the Accounts app.
- The **Export Selected Samples** function available from the Report and Graph screen overflow menus creates a .csv that can be emailed, sent to a network folder or saved to a FAT32 formatted USB flash drive.
  - When the export function is used, the data is **not** deleted from the database and can be accessed using the Data app.
  - Only one Kinetics run at a time may be added to a Report for export from the Data app.



- The **Archive Selected Samples** function available from the Run and Report screen overflow menus creates a .csv file with sample data that can be exported to a folder entitled ds11 on a FAT32 formatted USB flash drive or saved to a network folder.
  - When the archive function is used, the data is *permanently* deleted from the instrument database and cannot be restored to the instrument.
  - Multiple Kinetics runs may be archived in a single action.
- The archiving privilege for standard accounts is enabled or disabled using the Settings app. When disabled, only administrative accounts may archive data.
- The storage capability of the DS-11 FX is sufficient for several years of data for a lab with average use. Therefore, it is recommended that the archiving function be used only as an infrequent method of deleting old data from the DS-11 database. Although archiving a large amount of data may take a considerable amount of time, the archive process continues to work even when the interface goes to sleep after 10 minutes of no user activity. Touch the screen to wake-up the user interface and update the progress message.
- Use the Cancel button to stop the archive button. Samples already copied up to the stop point may be deleted from the instrument at the user's discretion.



## **Settings App**

The Settings app may be found on the second Home screen page. Only administrator accounts may access and change settings.

The app provides access to the following functions:

## **Backup and Restore**

- The backup process exports a copy of all user-defined app settings, methods, dyes, sample types, email addresses, shared folder paths, user accounts and sample results to either a USB flash drive or a network folder.
- Backup files include a DeNovix specific extension and may not be opened on a computer. The files may be restored back onto the DS-11 at any time.
- Automatic backup reminders may be enabled using the checkbox on the Backup screen.
- The restore function is used to recover user defined app settings, methods, dyes, sample types, email addresses, shared folder paths, user accounts and all sample results saved at the time of the backup. There are two options:
  - **Recover All:** Restores user defined app settings, user entered methods, user added dyes and sample types, email addresses, shared folder paths and saved user accounts as well as previous sample results. All new methods, sample and dye types, emails addresses, network folders, user app settings and data results accumulated since the time of the back-up will be overwritten during the restore process.
  - **Recover User Info Only**: Use this option to recover settings, methods, dyes, sample types, email addresses, shared folder paths and user accounts. This option will not restore sample data or overwrite new data.



#### Date/Time

· Select manual or automatic date and time options.

#### Language

- Although the Android system provides translations for some routine functions in many languages, the majority of the app specific text will be available in a limited number of language options.
- The DS-11 number format is determined by the language selected.
- Exit all apps before changing languages.

#### **Network Information**

• The WiFi and Ethernet media access control addresses can be found under this setting.

#### Permissions

· This selection is used to enable the following options:

**Archive:** Enable all standard accounts, including the General Account, to archive (delete) data from the instrument. Refer to the

**Automatic Updates:** Use this selection to enable or disable automatic updates of the software.

**Network Folder.** Use this selection to enable or disable the general account and/or standard user accounts to set-up network folders for data export/archive and system backups.



## Sound

· Volume controls are available for media and timer alarms.

# Storage

• Displays storage statistics.

# WiFi

To enter static IP addresses :

- 1. Ensure the WiFi is on. Select WiFi from the Settings list.
- 2. Tap the **Add** icon on the right side of the top action bar.
- 3. Dismiss the keyboard using the chevron on the bottom action bar.
- 4. Select the Show Advanced Options checkbox.
- 5. Select Static using the IP settings drop-down.
- 6. Enter the appropriate information and tap **Save.**

# Ethernet

To enter static IP addresses :

- 1. Ensure the Ethernet cable is connected. Select **Ethernet** from the Settings list.
- 2. Tap the name of the connection. Use the drop-down menu to select **Static**. Enter the appropriate information and tap **Apply**.

Note: WiFi will take precedence over the Ethernet when both are connected.

Contact your local IT department if additional help setting up WiFi or Ethernet static IP addresses is needed.



## **Updater App**

This app is used to update all apps to the most current available versions. The latest updates are available at www.denovix.com. For instruments without internet access, a zip file with the latest updates may be downloaded from the website onto a FAT32 formatted USB flash drive.

#### Procedure

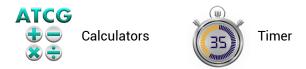
If the DS-11 is connected to the internet via WiFi or Ethernet proceed to step 3.

- 1. Download the required packages from the www.denovix.com website directly to the root level of the flash drive. Do not unzip the file folders.
  - The blue icon app requires both the Automated Updater (ds11current.zip) and the Latest Software Version (ds11latest.zip) packages.
  - The yellow icon app uses only the Latest Software Version (ds11latest.zip) package.
- 2. Insert the USB device into one of the ports located in the back of the DS-11.
- 3. Shut down all open apps.
- 4. Swipe to page 2 of the Home screen and launch the Updater app.
  - Blue icon version of the app:
    - Press the **Check for Updates** button. Follow the onscreen directions to Install the new yellow icon version of the Updater app.
    - Once the yellow Updater icon appears on the Home screen, relaunch the app to automatically update all other apps.
- 5. Click **OK** if a message is displayed that indicates new firmware is required. Do not turn off the power to the instrument during the 10- to 15 second firmware update process.

Note: Some installed apps are used for important background system functions and will not be visible on the Home screen.



# 18.Lab Tool Apps



#### **Calculator App**

Three calculators are included within the app. Use the tabs in the top action bar to navigate between the options.

#### **Oligo Calculator**

This calculator analyzes a user-inputed ssDNA or RNA sequence of interest.

- Outputs include:
  - Length
  - Molecular Weight (g/mol)
  - GC Content
  - Mass Extinction Coefficient (µg/OD @260 nm): This result may be copied and pasted into the Factor Input field used for nucleic acid concentration calculations in both the ssDNA and the Microarray apps.
  - Melting Temp: For sequences up to 13 nucleotides, the equation used is Tm= (wA+xT) \* 2 + (yG+zC) \* 4 where w,x,y,z are the number of the bases A,T,G,C in the sequence. For sequences longer than 13 nucleotides, the equation used is Tm= 64.9 +41\*(yG+zC-16.4)/(wA+xT+yG+zC). Both equations assume that the annealing occurs under the standard conditions of 50 nM primer, 50 mM Na+, and pH 7.0.



#### **Scientific Calculator**

Use this calculator for standard mathematical and scientific functions.

#### **Dilution Calculator**

This calculator is a useful tool to calculate how much of a stock solution should be added to a buffer solution to result in a final specified solution concentration.

• Both molar and mass units are available. However, conversions between the two types of units are not enabled.

# Timer App

This app is a lab tool that allows a user to set two independent timers.

- At the end of a specified period, an alarm will sound for a maximum of 90 seconds.
- Push the **Off** button to silence the alarm.
- The alarm can be acknowledged via the notification bar or by going back to the Timer app.
- The Timer will work in the background while other measurement apps are in use as well as when the screen is in the dark screen saver mode.



# 19. Maintenance

# Cleaning

Routine	1. Pipette 2 $\mu L$ of dH_20 onto the bottom sample surface. Lower the upper arm. Wait 1-2 minutes.
	2. Wipe away the water from both the upper and lower sample surfaces with a dry, lint-free lab wipe.
Between Measurements	Use a dry, lint-free lab wipe to remove samples from both the lower and upper sample surfaces.
Additional Cleaning	Use the following procedure to remove sticky solutions (e.g. proteins) that may have dried down on the sample surfaces.
	1. Pipette 2 $\mu L$ of 0.5 M HCl onto the bottom sample surface. Lower the upper arm. Wait 2-3 minutes.
	2. Wipe away the HCl from both the upper and lower sample surfaces with a dry, lint-free lab wipe.
	3. Repeat steps 1-2 with 2 $\mu L$ of $dH_20.$

## Guidelines

- Use only a dry, soft, lint-free cloth to clean the front screen.
- Do not use a spray bottle to apply water or any other solutions onto any surface of the instrument as the liquid may damage internal components.
- The use of detergents or isopropyl alcohol as cleaning solvents is not recommended as they may temporarily alter the hydrophobic nature of the sample surfaces.



#### ScreenLocker App

Launch the ScreenLocker app from page 2 of the Home screen prior to cleaning the touch screen. This will disable swiping motions from activating other software activities. Tap the Droid icon three times to unlock the screen.

#### **Cuvette and Fluorescence Mode Holder Cleaning**

The cuvette and FX tube holders may be cleaned of excess dust using canned air or a damp (not wet) lint free cotton swab.

- Do not allow any liquid to drip into the holders as it may damage the internal components of the instrument.
- · Refer to the cuvette manufacturer for guidance on cleaning cuvettes.

## **Solvent Compatibility**

The DS-11 spectrophotometer microvolume sample surfaces are compatible with most solvents typically used in life science laboratories.

Exception: Do not use Hydrofluoric Acid (HF) as the fluoride ion will dissolve the quartz fiber optic cable.

#### **Customer Support**

Contact DeNovix Customer Support at 1-302-442-6911 or <u>info@denovix.com</u> if additional help is required. Please include the serial number of your instrument when contacting Customer Support by email.

Outside of the US, please contact your local distributor for assistance.



# 20. Diagnostics



Although the DS-11 FX propriety SmartPath® technology enables accurate microvolume pathlength control and eliminates the need for routine recalibration, labs may choose to run the Diagnostics app to verify that the instrument is working within specifications.

## **DS-11 Self Test**

- Ensure that both the upper and lower microvolume measurement surfaces are clean prior to starting the Self Test microvolume mode assessment.
- For DS-11+ models, remove cuvettes from the holder block and ensure the light path is clear of all obstructions prior to starting the Self Test cuvette mode assessment.
- A spectral plot of the xenon flash lamp intensity and a table that compares measured versus specified values for a panel of specifications will be displayed. If any of the parameters fail to meet specifications, clean the measurement surfaces as described in the chapter entitled "Maintenance" and repeat.

#### Lamp Reset

The Lamp Reset function should only be used when there is concern that a method optimization was done on dirty sample measurement surfaces.



# **Microvolume Pathlength Verification**

The DeNovix LC-NA verification fluid is supplied as single use vials composed of a non-toxic solution of aqueous nicotinic acid. The solution is used to verify that the microvolume pathlengths are within specifications.

#### Procedure

- 1. Ensure both sample measurement surfaces are clean. Refer to the general cleaning instructions in the chapter entitled "Maintenance" for more information.
- 2. Launch the **Diagnostics** app and navigate to the **Verification** tab.
- 3. Enter the lot specific Target Absorbance stated on the ampule in the appropriate field.
- 4. Add 1 µL of dH<sub>2</sub>O to the pedestal, lower the arm and click **Blank**.
- 5. Wipe the upper and lower pedestals using a dry, lint-free laboratory wipe.
- 6. Vigorously shake the nicotinic acid ampule to thoroughly mix the solution. Ensure all of the solution is in the bottom portion of the ampule before opening.
- 7. Pipette 1µL of the solution onto the sample surface, lower the arm and click **Measure**.
- 8. Wipe the sample off both the top and bottom sample surfaces.
- 9. Repeat steps 7 and 8 for a total of 5 measurements using fresh aliquots for each measurement.

# Verification Check Troubleshooting

lssue	Probable Causes/ Solutions
Low Absorbance Values	Dirty measurement surfaces or improper Blank. Clean the surfaces, measure a new Blank using fresh source of dH <sub>2</sub> 0 and restart the procedure using a new ampule of LC-NA.
High Absorbance Values	Multiple measurements made using the same aliquot. Use a fresh aliquot for each measurement.
	Pipette tips not changed between aliquots. Use a fresh tip to pipette each aliquot onto the sample surface.
	The sample surfaces are not adequately cleaned between measurements. Wipe both the top and bottom surfaces with a dry lab wipe.
	The solution has concentrated due to prolonged exposure. Use a fresh ampule of solution.
High Standard Deviations	Multiple measurements made using a single 1 ul aliquot. Use a fresh aliquot for each measurement.
	Pipette tips not changed between aliquots. Use a fresh tip to pipette each aliquot onto the sample surface.
	The sample surfaces are not adequately cleaned between measurements. Clean both the top and bottom surfaces between measurements.



#### **Single Use Ampules**

- The LC-NA solution is supplied in single use ampules that should be used immediately upon opening. Significant changes in concentration and possible verification check failures may occur if the ampule is opened for longer than 1 hour prior to use. Store unopened ampules in a dark, room temperature environment.
- Always use a fresh ampule for each verification check procedure. Do not use parafilm or other methods of sealing an ampule as evaporation may still occur and result in verification test result errors.

Note: Only the LC-NA standard available from DeNovix and its authorized distributors should be used for the verification check. No other sources of nicotinic acid are tested and validated for use with the DS-11 verification procedure.

#### Results

A Pass or Fail message will appear in the results box at the bottom of the screen. If the instrument fails, please clean the surfaces and repeat the test with a fresh aliquot of LC-NA.

#### **Technical Support**

Contact DeNovix Customer Support if additional help is required. For US customers, please email the results directly to <u>info@denovix.com</u> using the Diagnostics app Overflow **Tech Support** function. Include your name, phone number, institute/company, and return email address. Please note responses will not be sent directly to the instrument email account.

The Tech Support email includes encrypted files for the DeNovix technical support team.

Outside of the US, please contact your local distributor for assistance.



# **FX-**Diagnostics

The FX diagnostics feature can be used for instruments with the integrated FX function or for FX USB accessory module attached to a DS-11 unit.

#### Procedure

- 1. Ensure FX sample measurement tube holder is empty and the lid is closed
- 2. Launch the FX Diagnostics app and push the Start button.
  - The software tests that each LED is firing and each detector is functioning properly. Results will be reported as Pass/Fail.

# **Technical Support**

Contact DeNovix Customer Support if additional help is required. For US customers, please email the results directly to <u>info@denovix.com</u> using the FX-Diagnostics app Overflow **Tech Support** function. Include your name, phone number, institute/company, and return email address. Please note responses will not be sent directly to the instrument email account.

The Tech Support email includes encrypted files that can only be opened by DeNovix technical support team.

Outside of the US, please contact your local distributor for assistance.



# 21. Troubleshooting

# **Quick Help**

Most issues concerning *accuracy, reproducibility, negative spectra* and *low nucleic acid purity ratios* are sample or technique related and are resolved by following the suggestions below:

- Establish a new Blank using the appropriate buffer.
- Ensure that the sample isolation procedure is optimized and that samples are purified when required prior to making absorbance measurements.
- Ensure all solutions are homogenous and well-mixed prior to sampling.
- Ensure sample concentrations fall within the absorbance limits of the instrument. In the case of fluorescence assays, ensure sample concentrations fall within the reagent limits as described by the assay manufacturer.

#### **Microvolume Mode**

- Ensure both top and bottom microvolume measurement surfaces are clean prior to making the Blank measurement.
- Always remove sample solutions from both the top and bottom measurement surfaces using dry, lint-free wipe immediately after each measurement is complete.
- Use calibrated pipettors and properly fitting tips to ensure a full 1 µL aliquot is delivered to the sample measurement surface. Protein samples sometimes wick up on the outside of the tip and may not be properly dispensed.
- Use a clean pipette tip and a new aliquot of sample for each microvolume measurement.



#### **Cuvette Mode**

- Use cuvettes with Z heights of 8.5 mm.
- Use quartz or UV transparent cuvettes for UV range measurements.
- Ensure cuvettes are clean prior to use.
- Fill cuvettes to manufacturer's specifications.
- Confirm the mode selection has been changed on the Run screen to the proper cuvette pathlength prior to a measurement.
- Ensure cuvettes are properly inserted following the etched arrow light path.
- Refer to a reagent kit manufacturer for troubleshooting colorimetric assays.

#### **Fluorescence Mode**

- Use 200 µL volumes in a clear thin walled PCR tube
- Avoid introducing air bubbles into the sample solution when mixing samples.
- Ensure all sample concentrations in the thin walled assay tubes fall within the limits of the assay or reagent kit for accurate results.
- Follow assay reagent manufacturers' recommendations regarding temperature, incubation time, and protection from ambient light.
- Follow assay reagent manufacturers' recommendations regarding suggested standard curve concentrations and data analysis.
- Confirm that the correct LED excitation source was used for all user-defined assays and methods.
- Confirm that standard concentrations and dilutions were performed correctly.
- Confirm that the correct concentration units for the standard curve and the unknown samples were used to calculate the stock concentrations.



- Ensure that the correct optional dilution factor (or uL added in the case of Qubit<sup>®</sup> assays) is entered into the appropriate Run screen field before a measurement is made.
- Ensure all samples and standards are treated identically in terms of incubation times and temperature.
- Minimize assay tube and solution temperature fluctuations as these may impact the accuracy of the measurement.
- Do not label the side of an assay tube as this could interfere with the sample measurement.

Additional troubleshooting resources (technical notes and FAQ's) are available at www.denovix.com.

## **Software Errors**

Pop-up messages will provide guidance for software-based errors or invalid user actions. In most cases, simply relaunching the app will allow the user to continue to make measurements. Update the software using the **Updater** app if experiencing a reoccurring software error.

Contact DeNovix Customer Support 302-442-6911 or info@denovix.com if additional help is required. Please include the serial number of your instrument when contacting Customer Support by email. The serial number may be found on the bottom of the instrument.

Outside of the US, please contact your local distributor for assistance.

#### **Printer and Barcode Scanner**

The DS-11 FX is compatible with selected DYMO printers\* and Opticon OPI 3601 USB barcode scanners. For accessory product troubleshooting and warranty information, please contact the manufacturer of the product. DeNovix support is limited to the specific use of the product with a DS-11 FX instrument.

\*Please refer to <u>www.denovix</u> for a list of supported Dymo printers.

# 22. Customer Support

DeNovix Inc. 3411 Silverside Road, Hanby Building Wilmington, DE USA 19810 302-442-6911 info@denovix.com www.denovix.com

Please include the serial number of your instrument when contacting Customer Support by email. If sending Customer Support a Screen Capture or a report .csv file using the DS-11 email feature, include your name, institute/company, phone number and return email address. **Please note responses will not be sent directly to the email account used by the instrument.** 

Outside of the US, please contact your local distributor for assistance.

#### Warranty

All product(s) and accessories sold by DeNovix Inc. are under warranty against manufacturing defects in parts for the 36 month period from the date of delivery.

#### Disclaimer

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