DeNovix dsDNA High Sensitivity Assay

Technical Note 145

Introduction

The DeNovix dsDNA High Sensitivity Assay enables accurate detection of purified double-stranded DNA (dsDNA) samples with a standard detection range from 100 pg to 250 ng total mass in 200 µL volumes. This equates to sample concentrations of 10 pg/µL to 250 ng/µL when using between 1-20 µL sample volumes in 200 µL assay volumes. The assay is linear for sample concentrations as high as 250 ng/µL when adjusting volumes to 1 µL of sample into 199 µL of working reagent. Total mass should not exceed 250 ng for best results.

Extended Range

The lower detection limit can be extended down to 5 pg/µL sample by adding 20 µL of the 5 pg/µL sample to 180 µL of the working reagent.

Kit Contents

Three assay sizes are available. The volume of components in each kit are sufficient for 1000, 250, and 50 (evaluation size) assays respectively. Kit components are shown in the table below.

Component	1000	250	Eval
DeNovix dsDNA High Sensitivity Dye (100x)	2 x 1 mL	0.5 mL	100 µL
DeNovix dsDNA High Sensitivity Buffer	200 mL	50 mL	10 mL
25 ng/µL dsDNA Standard (calf thymus)	0.5 mL	0.5 mL	0.5 mL
0 ng/µL dsDNA Standard	0.5 mL	0,5 mL	0.5 mL

Store the kit at 4° C and protect the dye solution from light. The kit is stable for 12 months from ship date when stored as recommended.

The dye is a potentially harmful chemical. Exercise universal laboratory safety precautions when handling the dye and dispose of the dye as hazardous chemical waste according to your local regulations.

Instrument Compatibility

The DeNovix dsDNA High Sensitivity quantitation assay is designed for use with fluorometers or fluorescence plate readers equipped with excitation and emission filters for detecting green fluorescence. The unique spectral properties of the kit dye make it especially well-suited for use with instruments with blue LED excitation sources.

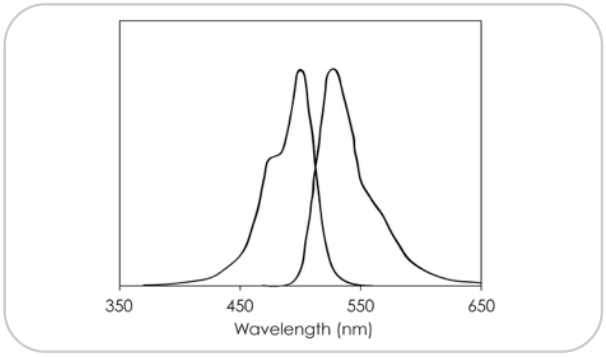

Fig. 1: Excitation and emission spectra for DeNovix dsDNA Broad Range quantitation reagent in the presence of excess dsDNA.

The kit is compatible with fluorescence microplate readers and fluorometers with the appropriate excitation sources and emission detectors.

Specific instructions (Technical Note 144) using the 2 point standard assay with DeNovix DS-11 FX , FX module or the QFX fluorometer are available at www.denovix.com.

Assay Considerations

Calf thymus DNA is provided as the reference standard as it is double-stranded, highly polymerized and is approximately 58% AT (42% GC). At times it may be preferable to use a dsDNA standard similar to the unknown samples (i.e. similar in size, linear vs. circular). For bacterial DNA, consider using a species-specific standard as the GC content varies widely depending on the species.

Although many instruments including the DeNovix DS-11 FX and QFX fluorometers offer the option to use previously saved values, it is recommended that a new standard curve be generated at the time of the assay for optimal results.

Assay Detection Limits

Fluorescent quantification specifications are often expressed in a variety of conventions. The full detection range (including the extended range) of this assay can be expressed in the following specifications:

Specification	Range
Absolute mass per assay tube	100 pg to 250 ng per 200 µL
Concentration in sample stock tube	5 pg/µL to 250 ng/µL

Best Practices

It is important to pay careful attention to pipetting accuracy and overall sample handling techniques when quantitating picogram amounts of dsDNA.

- Use properly calibrated pipettes and DNase-free pipette tips. Use the smallest calibrated pipettor available to dispense each sample volume.
- Use thin-walled, clear 0.5 mL PCR tubes for assay measurements (DeNovix cat# TUBE-PCR-0.5-500 or equivalent) or black-walled microplates. If using tubes, label only the top, not the sides of the tube.
- Avoid introducing air bubbles into the sample solution when mixing samples.
- Prepare the working solution fresh for each assay. Discard the solution after 24 hours.
- Ensure all samples and standards are treated identically in terms of incubation times and temperature.
- Although standard curves may be saved and re-used, it is recommended that a new curve be generated for each assay.
- Ensure all sample concentrations in the assay tubes or microplate wells fall within the limits of the reagent kit for accurate results.

Assay Prep

- Allow all solutions to equilibrate to room temperature before use.
- Vortex, then centrifuge vials briefly before opening to minimize reagent loss on the cap.

Assay Protocol

1. Prepare 200 µL of working solution for each standard and sample to be tested by diluting the dye 1:100 in the assay buffer. Mix well before use.
2. For each standard or unknown sample, add 190 µL of the working solution per tube or micro well. Adjust volume when adding more or less than 10 µL of the unknown sample.
3. Add 10 µL of each standard and between 1-20 µL of the unknown DNA sample to the assay tube or micro well and mix well.
4. Incubate standards and samples at room temperature for 5 minutes. Protect from light. The assay is stable for 4 hours at room temperature.
5. Generate the standard curve and then measure the samples using the proper excitation source and emission filters. The DeNovix DS-11 FX or QFX software automatically utilizes the correct excitation and emission set-up.

Standard Dilutions

Preparing diluted standards is not required when using the optimized preconfigured 2 point assay option in the DeNovix FX or QFX software. For the DeNovix User Defined Standards option or for use on microplate readers, prepare a set of DNA standards by serial dilution of the 25 ng/µL standard in 1X TE buffer (10 mM Tris pH 7-8, 1 mM EDTA) as shown in the table below.

Standard	DNA	TE
25 ng/µL	100 µL of 25 ng/µL stock tube	None
10 ng/µL	40 µL of 25 ng/µL standard	60 µL
2.5 ng/µL	25 µL of 10 ng/µL standard	75 µL
1 ng/µL	40 µL of 2.5 ng/µL standard	60 µL
0.25 ng/µL	25 µL of 1 ng/µL standard	75 µL
0.1 ng/µL	40 µL of 0.25 ng/µL standard	60 µL
0.03 ng/µL	30 µL of 0.1 ng/µL standard	70 µL
0 ng/µL	100 µL of 0 ng/µL stock tube	None

Data Analysis

Sample concentrations are automatically calculated when using a DeNovix DS-11 FX or QFX fluorometer. For all other instruments, follow the instructions below:

1. Generate a standard curve to determine the unknown DNA concentration.
2. Average the triplicate values for each sample and subtract the average zero DNA value from each data point.
3. Plot the fluorescence RFU values for the DNA standards on the y-axis and ng/well DNA on the x-axis, and fit a trend line (Figure 2) through these points to generate a standard curve with a y-intercept = 0.
4. Use the equation for the trend line to calculate the amount of unknown DNA in each well (y = fluorescence and x = ng DNA per well or tube).

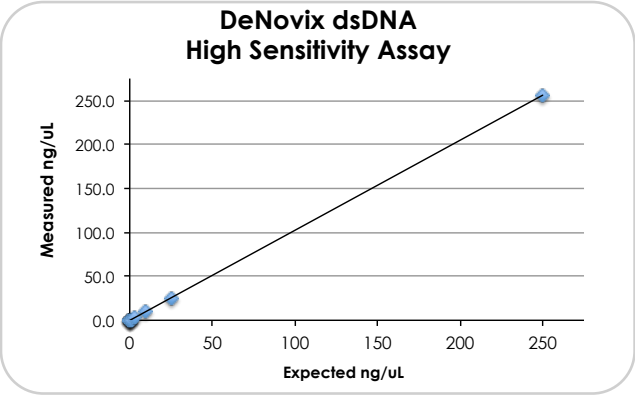

Fig. 2. Calf Thymus DNA measured using the DeNovix dsDNA High Sensitivity Assay on a DS-11 FX fluorometer.

Troubleshooting

- Confirm that the correct excitation source and emission filters were used at the time of the measurement.
  - Note: The DeNovix DS-11 and QFX software
  - automatically uses the correct LED and emission filter.
- 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.
- If applicable, ensure that the correct dilution factor or sample volume added value is entered into the appropriate Run screen field before a measurement is made.

Appendix: Solvent Compatibility

Compound	Maximum concentration in 200 µL assay	Signal Decrease
Ammonium Acetate	50 mM	14%
Sodium Chloride	50 mM	14%
Magnesium Chloride	5 mM	16%
Sodium Acetate	30 mM	11%
Ethanol	1%	21%
Phenol	0.1%	11%
Chloroform	1%	34%
SDS	0.01%	31%
SDS	0.001%	9%
Trtion X-100	0.001%	20%
dNTPs	100 µM	11%

DNA standards assayed in the absence or presence of contaminants listed at the concentrations above. A result of OK indicates that there was <20% change in the signal in the absence of the contaminant. *Mix of dATP, dCTP, dGTP, dTTP.

The DeNovix dsDNA Broad Range and Ultra High Sensitivity assays are more tolerant of some contaminants compared to the High Sensitivity assay reagents. If the High Sensitivity assay is not compatible with your dsDNA extraction procedure, consider using an alternate DeNovix dsDNA assay kit. For comparison, the standard detection ranges of the three assays are as follows:

DeNovix dsDNA Assay	Range
Broad Range	100 pg/µL to 2000 ng/µL
High Sensitivity	10 pg/µL to 250 ng/µL
Ultra High Sensitivity	0.5 pg/µL to 300 pg/µL

Instructions specific to performing a 2 Point standard curve assay on a DeNovix fluorometer (Technical note 144) is available at www.denovix.com

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