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Product Information

AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit with 7 DNA Standards

Catalog Number:

31028-T AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit, trial size
31028 AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit with 7 DNA Standards

Kit Contents

Component	31028-T 200 assays	31028 1000 assays
99977: AccuClear dye (100X in DMSO)	0.5 mL 99977-T	2 X 1 mL 99977
99978: AccuClear buffer	50 mL 99978-T	200 mL 99978
dsDNA Standard in 10 mM Tris pH 7.5, 1 mM EDTA, 2 mM sodium azide 25 ng/uL dsDNA from calf thymus	1 mL 31029C	N/A
dsDNA Standards in 10 mM Tris pH 7.5, 1 mM EDTA, 2 mM sodium azide 0.03, 0.1, 0.3, 1, 3, 10, and 25 ng/uL dsDNA from calf thymus	N/A	Set of 7, 0.5 mL each 31028C

Storage and Handling

Store kit at 4°C. Protect dye from light. The kit is stable for at least 6 months from date of receipt when stored as recommended. AccuClear 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.

Spectral Properties

Ex/Em: 468/507 nm (bound to dsDNA). See Figure 1 for spectra.

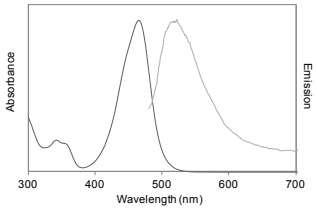
Product Description

AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kits provide highly sensitive and accurate DNA quantitation across a broad range of DNA concentrations. Unlike absorbance-based measurements, AccuClear™ dye is highly selective for double-stranded DNA over single stranded DNA or RNA (Figure 2). The assay is linear between 30 pg and 250 ng of dsDNA per assay (3 pg/uL to 25 ng/uL sample concentration) in microplate format (Figure 3).

The AccuClear Ultra High Sensitivity dsDNA quantitation assay is designed for use with fluorescence 96-well plate readers equipped with excitation and emission filters for detecting green fluorescence. The unique spectral properties of AccuClear dye make it especially well-suited for use with instruments with blue LED excitation sources. Biotium's AccuLite™ 470 handheld fluorometer is pre-programmed for use with the AccuClear assay. AccuClear also is compatible with handheld fluorometers such as Invitrogen's Qubit® and Promega's QuantiFluor™-P, however the standard curve calibration programs for these instruments may not cover the full dynamic range of the AccuClear kit standard curve.

Biotium offers AccuBlue Broad Range, AccuBlue High Sensitivity, and AccuClear Ultra High Sensitivity Assays in a variety of sizes, with or without dsDNA standards (see related products).

Figure 1. Absorbance and emission spectra of AccuClear dye bound to dsDNA.



Assay Protocol

Note: see the Appendix for information on using the AccuClear assay with the AccuLite 470 fluorometer.

1. Use properly calibrated pipettes and DNase-free pipette tips, tubes and plates for best accuracy. It is recommended to test each DNA standard and each unknown sample in triplicate. If more than one 96 well plate is to be tested in a single assay, it is recommended to include a standard curve on each plate to minimize variability between plates. See Considerations for Data Analysis (next page) for more information on standards.

2. Warm all components to room temperature before use. AccuClear dye is provided in DMSO, which may freeze during storage at 4°C. You can place all kit components in a 37°C water bath for rapid warming; be sure to allow solutions to cool to room temperature before using. Before removing the required volume, mix each component well by shaking or vortexing, and centrifuge vials briefly before opening to minimize reagent loss on the cap.

3. For 31028 (1000 assays), use the set of DNA standards provided. For 31028-T, trial size (200 assays), prepare a set of DNA standards by diluting the 25 ng/uL standard in 1X AccuClear buffer as shown in Table 1. Volumes may be scaled as necessary. The two lowest concentration DNA dilutions should be prepared fresh on the day of assay. The other DNA dilutions can be stored at 4°C for at least 6 months with the addition of 2 mM final concentration sodium azide.

Table 1. Preparation of DNA standards for 31028-T (trial size kit)

Concentration	DNA	1X AccuClear buffer
25 ng/uL	100 uL of 25 ng/uL	--
10 ng/uL	40 uL of 25 ng/uL	60 uL
3 ng/uL	12 uL of 25 ng/uL	88 uL
1 ng/uL	10 uL of 10 ng/uL	90 uL
0.3 ng/uL	10 uL of 3 ng/uL	90 uL
0.1 ng/uL	10 uL of 1 ng/uL	90 uL
0.03 ng/uL	10 uL of 0.3 ng/uL	90 uL
0.01 ng/uL	10 uL of 0.1 ng/uL	90 uL
0.003 ng/uL	10 uL of 0.03 ng/uL	90 uL
0 ng/uL	0 uL	100 uL

4. On the day of the assay, prepare 200 uL of working solution for each sample to be tested. Dilute the dye at a ratio of 1:100 in buffer in a plastic container and mix well by vortexing or shaking. For example, mix 200 uL of dye with 20 mL assay buffer to prepare enough working solution for an entire 96 well plate. Volumes can be scaled as required. Working solution is stable for 24 hours.

5. For each sample to be tested, pipette 200 uL of the working solution per well of a black 96-well microplate. To test samples in triplicate, prepare three separate wells for each DNA standard and three separate wells for each unknown DNA sample. Accurate multi-channel pipettes and reagent reservoirs can be used to increase throughput. Black plates are recommended to minimize fluorescence bleed-through between wells. We have found that black 96-well plates from Greiner Bio One or Corning give the most consistent signal-to-noise ratio at low DNA concentrations.

6. Add 10 uL of each dsDNA standard and unknown into its own separate well containing working solution and mix well by pipetting up and down.

7. Incubate the microplate at room temperature for 5 minutes in the dark. The assay plate is stable for 4 hours at room temperature.

8. Measure fluorescence using a microplate reader to set to 468 nm excitation/507 nm emission maxima or other filter combination for detecting green fluorescence (e.g., FITC filter set).

9. Generate a standard curve to determine the unknown DNA concentration (see Figure 2). Average the triplicate values for each sample and subtract the average 0 ng DNA value from each data point. Plot the fluorescence values for the DNA standards on the y-axis and ng/well DNA on the x-axis, and fit a trend line through these points to generate a standard curve with a y-intercept = 0. Use the equation for the standard curve trend line to calculate the amount of unknown DNA in each well (y = fluorescence and x = ng DNA per well). Note: the standard curve shown in Figure 2 is for reference only. You must generate your own standard curve using your instrument to calculate the amount of DNA in your unknown samples.

Considerations for Data Analysis

Calf thymus DNA can serve as a reference for most plant and animal DNA because it is double-stranded, highly polymerized and is approximately 58% AT (42% GC). Lambda dsDNA yields similar results (Figure 2). You may wish to use a standard similar to your unknown samples in DNA length, structure (i.e., linear vs. circular), or GC content. For bacterial DNA, a species-specific standard may be desired because the GC content varies widely depending on the species. The AccuClear™ dsDNA quantitation assay is available without standards (catalog no. 31027) for customer who wish to prepare their own standards.

The linear range of the AccuClear assay extends from 250 ng to 0.03 ng. The standard curve can be extended to 300 ng with some loss of linearity. If lower end standards are desired, you can prepare 0.01 ng/uL and 0.003 ng/uL standards by diluting the 0.1 ng/uL and 0.03 ng/uL DNA 1:10 in AccuClear buffer. Use 10 uL of these standards in the assay to obtain 0.1 ng and 0.03 ng data points. It is recommended to prepare the 0.01 ng/uL and 0.003 ng/uL standards fresh on the day of assay.

If the fluorescence of any of the unknown samples is higher than the linear range, further dilute the sample and add 10 uL of the diluted sample to perform the assay. For consistency, it is best to use the same volume of sample in all the wells.

Due to differences in instruments, check instrument settings to optimize for the best linearity. Some factors that can affect the final linearity and relative fluorescence intensity are: (1) the excitation and emission wavelengths and bandwidths, (2) cut-off filters, (3) sensitivity settings, (4) pipetting accuracy, and (5) microplate manufacturer.

The effects of common DNA contaminants such as salts, solvents, detergents and protein on the AccuClear assay are listed in Table 2. Please also see our AccuBlue™ dsDNA Quantitation Assays (related products), which are more tolerant of some contaminants compared to AccuClear.

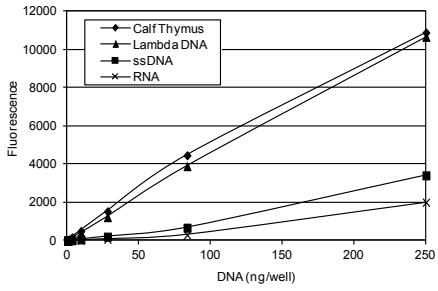


Figure 2. Selectivity of AccuClear dsDNA quantitation assay for double-stranded DNA compared to single stranded DNA and single-stranded RNA.

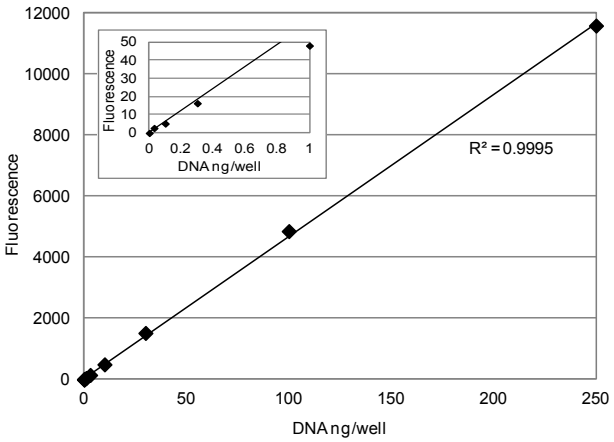


Figure 3. Linearity of AccuClear Ultra High Sensitivity dsDNA quantitation assay between 30 pg and 250 ng per well in microplate assay with excitation/emission at 468/507 nm. The inset shows the lower portion of the curve. Note: the graph shown above is for reference only. You must generate your own standard curve using your instrument to calculate the amount of DNA in your unknown samples.

Table 2. Effect of common DNA contaminants on AccuClear assay signal

Compound	Initial concentration in DNA sample	Final concentration in assay (200 uL)	Decrease in Signal
Sodium Chloride	1 M	50 mM	14%
Magnesium Chloride	100 mM	5 mM	16%
Sodium Acetate	600 mM	30 mM	11%
Ammonium Acetate	1 M	50 mM	14%
Ethanol	20%	1%	21%
Phenol	2%	0.10%	11%
Chloroform	20%	1%	34%
SDS	0.2%	0.01%	31%
SDS	0.02%	0.001%	9%
Triton X-100	0.2%	0.01%	36%
Triton X-100	0.02%	0.001%	20%
Tween-20	0.1%	0.005%	20%
BSA	20 mg/mL	1 mg/mL	36%
dNTPs	2 mM	100 uM	11%

Related Products

Catalog number	Product
E90001	AccuLite™470 Mini Fluorometer
31029	AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit with 1 DNA Standard (2000 assays)
31027	AccuClear™ Ultra High Sensitivity dsDNA Quantitation Solution (1000 assays)
31006	AccuBlue™ High Sensitivity dsDNA Quantitation Kit
31007	AccuBlue™ Broad Range dsDNA Quantitation Kit
41003	GelRed™ Nucleic Acid Gel Stain, 10,000X in water
31003-T	Fast EvaGreen® qPCR Master Mix, trial size
31020-T	Fast Plus EvaGreen® qPCR Master Mix, trial size

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF™dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

Appendix: AccuClear Assay Protocol for the AccuLite 470 Fluorometer

Sample Preparation

Note: if using Mini Glass Tubes, 100 uL sample volume can be used. Scale all volumes in the reaction (working solution and DNA) proportionally.

1. Prepare working solution as described in the AccuClear protocol.
2. For each sample to be tested, pipette 200 uL of the working solution into a 0.2 mL thin-walled clear PCR tube. To test samples in triplicate, prepare three tubes for each sample. Prepare two additional tubes for standards.
3. Prepare standards. Only the 0 ng DNA standard (blank) and 100 ng DNA standard are required. Pipette 10 uL of AccuClear Buffer into the 0 ng DNA tube (blank). Pipette 10 uL of the 10 ng/uL DNA standard into the 100 ng DNA tube. Pipette up and down or vortex to mix.
4. Prepare samples by pipetting 10 uL of each sample DNA per tube. Pipette up and down or vortex to mix.

Calibration

To move to a previous screen at any time, select Return. Continue selecting Return to go back to the Main Menu.

1. From the AccuLite Main Menu, select Calibrate.
2. Select Accu dsDNA from the assay list.
3. Insert the blank tube and close the cover. Select Blank.
4. After blanking, the standard value will appear (00100.000). Insert the 100 ng DNA standard tube and close the cover. Press Measure.
5. Calibration Finished will appear on the screen.
6. Select Return to return back to the Main Menu.

Sample Measurement

1. From the AccuLite Main Menu, select Measure.
2. Select Accu dsDNA from the assay list.
3. Insert the first sample tube and close the cover. Select Measure. The value shown is ng DNA per tube.
4. Select Save to save the data in the meter.

   Alternatively, you can manually the record data without saving, then select Return.
5. Insert next sample and select Measure.
6. After reading all samples, select Return repeatedly to navigate back to main menu.

Retrieving Saved Data

1. From the AccuLite Main Menu, select Data.
2. Select Accu dsDNA from the assay list.
3. Use the arrow keys to navigate through saved data points. Data points are numbered (##) in order of measurement.
4. To erase data, select Erase All and Confirm.
5. To return to previous screens, select Return.

Performing a Full Calibration Curve with AccuLite

The first time you perform the assay, or if unexpected results are obtained, you may wish to perform a full calibration curve to verify that the assay is performing properly. In this case, perform the 2 point calibration as described above, then read the full set of standards as if they were unknown samples. Plot the standard curve as described in the AccuClear protocol.

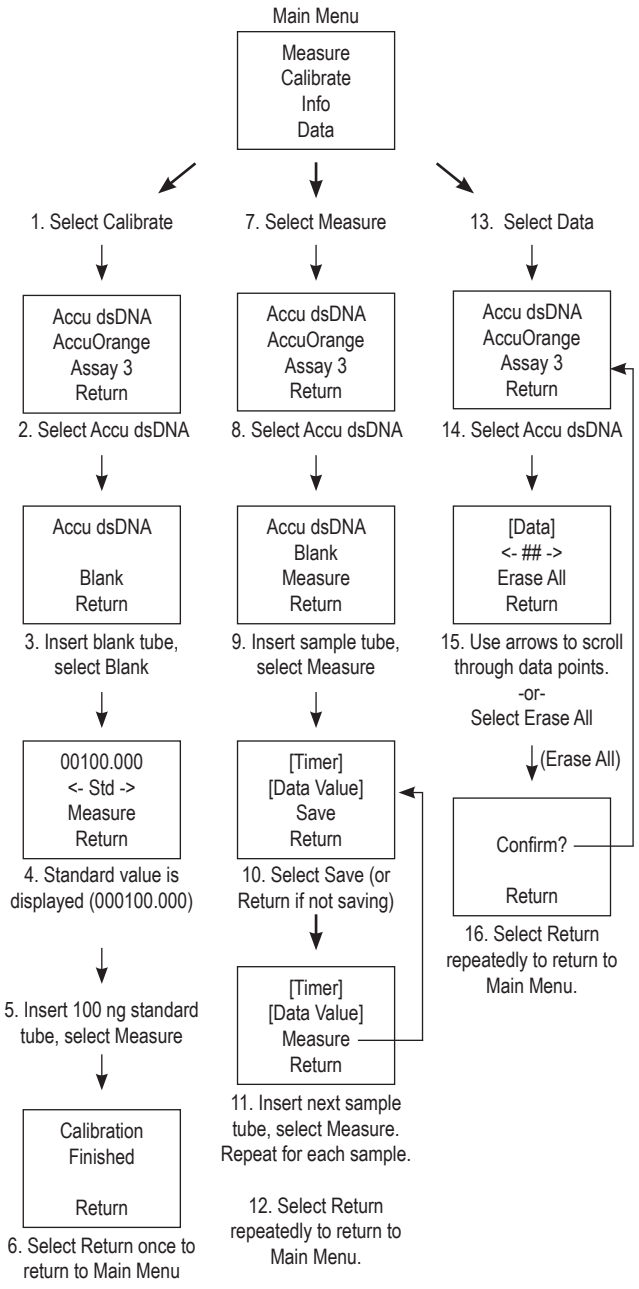


Figure 4. AccuLite user menu tree showing AccuClear calibration, measurement, and data retrieval steps. See the AccuLite user manual for complete user menu tree.