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Product Information

AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit with 1 DNA Standard

Catalog Number: 31029

Unit Size: 4000 assays (200 uL microplate assay)

Kit Contents

Component	Size
99977: AccuClear™ dye, 100X in DMSO	4 X 1 mL
99979: 20X AccuClear™ buffer	50 mL
31029C: AccuClear™ DNA Standard 25 ng/uL calf thymus DNA in TE buffer	3 X 1 mL

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 (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. Unlike absorbance-based measurements, AccuClear™ dsDNA Quantitation dye is highly selective for double-stranded DNA over single stranded DNA or RNA (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. AccuClear™ 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.

The AccuClear™ Ultra High Sensitivity dsDNA Quantiation Kit with 1 DNA Standard contains AccuClear™ dye, 20X assay buffer, and a 25 ng/uL calf thymus dsDNA standard stock solution that can be used to prepare a set of standards. Biotium also offers the AccuClear™ Ultra High Sensitivity dsDNA Quantiation Kit with 7 DNA Standards (1000 assays, catalog no. 31028), which includes AccuClear™ dye, 1X assay buffer, and a set of prediluted calf thymus dsDNA standards. AccuClear™ Ultra High Sensitivity dsDNA Quantitation Solution (1000 assays, catalog no. 31027), which includes dye and 1X buffer without standards, is available for customers who wish to prepare their own DNA standards.

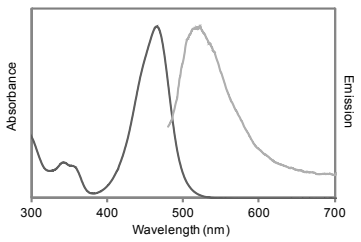

Figure 1. Absorbance and emission spectra of AccuClear™ dye bound to dsDNA.

Assay Protocol

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. Prepare 1X AccuClear™ buffer by diluting the 20X buffer solution 1:20 in dH_2O. 1X AccuClear™ buffer can be stored at 4°C for at least 6 months with the addition of 2 mM final concentration sodium azide.

4. Prepare a set of DNA standards by diluting the 25 ng/uL standard in 1X AccuClear™ buffer as shown below. Volumes may be scaled as necessary. The two lowest concentration DNA dilutions (H, I) should be prepared fresh on the day of assay. The other DNA dilutions (B-G) can be stored at 4°C for at least 6 months with the addition of 2 mM final concentration sodium azide.

Standard	Concentration	DNA	1X AccuClear buffer
A	25 ng/uL	31029C AccuClear DNA Standard, 25 ng/uL stock	--
B	10 ng/uL	400 uL of A	600 uL
C	3 ng/uL	120 uL of A	880 uL
D	1 ng/uL	100 uL of B	900 uL
E	0.3 ng/uL	100 uL of C	900 uL
F	0.1 ng/uL	100 uL of D	900 uL
G	0.03 ng/uL	100 uL of E	900 uL
H	0.01 ng/uL	100 uL F	900 uL
I	0.003 ng/uL	100 uL of G	900 uL
J	0 ng/uL	0 uL	1000 uL

5. On the day of the assay, prepare working solution. Dilute the dye at a ratio of 1:100 in buffer in a plastic container (do not use a glass container) and mix well by vortexing or shaking. Prepare 200 uL of working solution for each sample to be tested. For example, mix 200 uL of dye with 20 mL 1X assay buffer to prepare enough working solution for an entire 96 well plate. Volumes can be scaled as required. Prepare only as much working solution as you plan to use within 24 hours, and discard unused working solution 24 hours after mixing.

6. 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.

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

Continued next page

8. Pipette 10 uL of each unknown DNA into its own separate well containing working solution and mix well by pipetting up and down.

9. Incubate the microplate at room temperature for 5 minutes in the dark.

10. 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). We recommend measuring fluorescence within 1 hour of performing the assay, because fluorescence signal will decrease over time after DNA and dye are combined (~15% decrease after 3 hours and ~30% decrease after 6 hours).

11. 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 3). 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 1. Please also see our AccuBlue™ dsDNA Quantitation Assays (related products), which are more tolerant of some contaminants compared to AccuClear™.

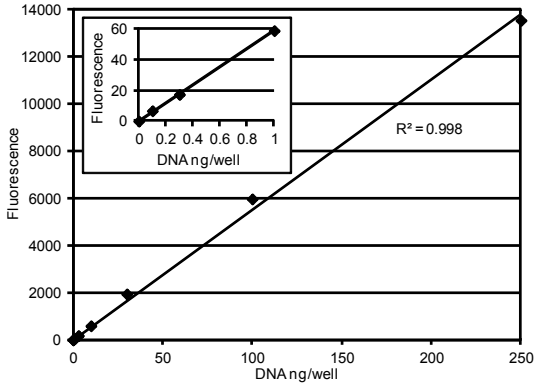

Figure 2. Linearity of AccuClear™ dsDNA quantitation assay between 30 pg and 250 ng per well in microplate assay with excitation/emission at 468/507 nm. The inset shows the lower portion of the curve.

Table 1. 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%
BSA	20 mg/mL	1 mg/mL	36%
dNTPs	2 mM	100 uM	11%

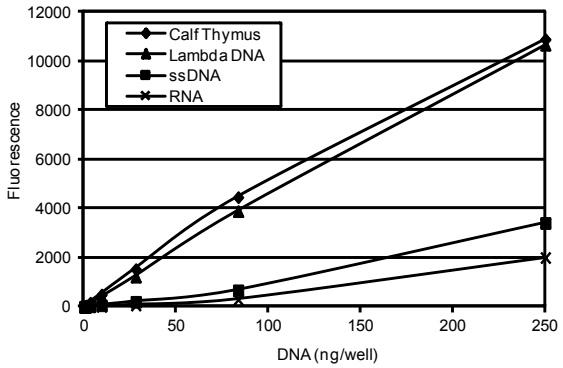

Figure 3. Selectivity of AccuClear™ dsDNA quantitation assay for double-stranded DNA compared to single stranded DNA and single-stranded RNA.

Related Products

Catalog number	Product
31027	AccuClear™ Ultra High Sensitivity dsDNA Quantitation Solution (1000 assays)
31028	AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit with 7 DNA Standards (1000 assays)
31006	AccuBlue™ High Sensitivity dsDNA Quantitation Kit with 8 DNA Standards
31007	AccuBlue™ Broad Range dsDNA Quantitation Kit with 9 DNA Standards
31008-T	AccuBlue™ High Sensitivity dsDNA Quantitation Solution, trial size
31009-T	AccuBlue™ Broad Range dsDNA Quantitation Solution, trial size
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.