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Product Information

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

Catalog Number and Kit Size
31029: 2000 assays

Kit Contents

Component	Size
AccuClear® dye (100X in DMSO)	99977 4 x 1 mL
DNA Quantitation Buffer, 20X Concentrate	99979-25mL 25 mL
dsDNA Standard, 25 ng/uL	31029C 3 X 1 mL

Storage and Handling
Store kit at 4°C. Protect dye from light. The kit is stable for at least 12 months from date of receipt when stored as recommended. AccuClear® dye is stable for storage at room temperature for up to 6 months. AccuClear® dye is a potentially harmful chemical. Exercise universal laboratory safety precautions when handling the dye.

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 0.03 ng and 250 ng of dsDNA per assay (3 pg/uL to 25 ng/uL sample concentration) in 96-well microplate format (Figure 3).

The AccuClear® Ultra High Sensitivity dsDNA quantitation assay is designed for use with 96-well plates and fluorescence microplate 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® 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.

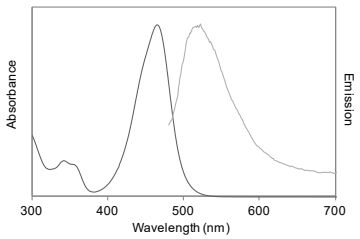

Figure 1. Absorbance and emission spectra of AccuClear® dye bound to dsDNA.

Assay Protocol
The first time that you use this kit, dilute the 20X buffer concentrate to 1X by adding dH_2O directly to the bottle containing the concentrated buffer. To 99979-25mL add 475 mL dH_2O. Invert the bottle several times to mix completely and mark the top of the cap to indicate that the buffer has been diluted to 1X.

1. Use black 96-well microplates (to minimize fluorescence bleed-through between wells), properly calibrated pipettes and DNase-free plasticware for best accuracy. It is recommended to test each DNA standard and each unknown sample in triplicate.

2. Warm all components to room temperature before use. AccuClear® dye is provided in DMSO, which may freeze during storage at 4°C. 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 a set of DNA standards by diluting the 25 ng/uL standard in 1X DNA Quantitation 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

Concentration	DNA	1X 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, protected from light.

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.

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. Use 1X DNA Quantitation Buffer or 1X TE as the zero standard.

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

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. To determine the unknown DNA concentration generate a standard curve (see Figure 3). Average the triplicate values for each standard sample and subtract the average zero 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 Solution is available without standards (cat. no. 31027) for customers who wish to prepare their own standards.

The linear range of the AccuClear® assay extends from 250 ng to 0.03 ng. 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.

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.

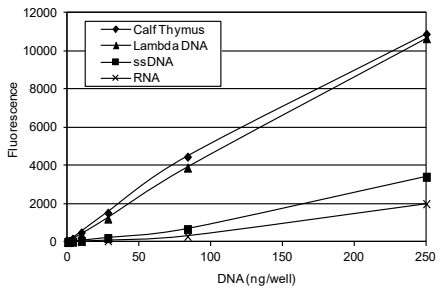

Figure 2. Selectivity of AccuClear® dsDNA quantitation assay for double-stranded DNA compared to single stranded DNA and single-stranded RNA.

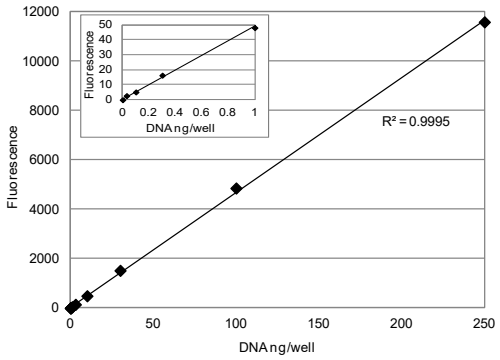

Figure 3. Linearity of AccuClear® dsDNA quantitation assay between 30 pg and 250 ng per well in microplate assay with excitation/emission at 468/507 nm. The inset shows the lower portion of the curve.

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%
Tween-20	0.1%	0.005%	20%
CTAB	0.01%	0.0005%	63%
BSA	2 mg/mL	0.1 mg/mL	30%*
dNTPs	2 mM	100 uM	11%

*0.1 mg/mL BSA in the assay resulted in a 30% decrease in peak fluorescence, and is not compatible with quantitation below 20 ng DNA in the assay.

Related Products

Catalog number	Product
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
31060	AccuBlue® NextGen dsDNA Quantitation Kit
31066	AccuGreen™ High Sensitivity dsDNA Quantitation Kit (for Qubit®)
31069	AccuGreen™ Broad Range dsDNA Quantitation Kit (for Qubit®)
31073	AccuBlue® Broad Range RNA Quantitation Kit
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in water
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in water
41029	GelRed® Agarose LE
41030	GelGreen® Agarose LE
31045, 31046	Forget-Me-Not™ EvaGreen® qPCR Master Mix
31041	Forget-Me-Not™ EvaGreen® qPCR Master Mix, 2-Color Tracking
31043	Forget-Me-Not™ Universal Probe Master Mix
CD201	RNAstorm™ Kit for Isolation of RNA from FFPE Tissue Samples
CD202	DNAstorm™ Kit for Isolation of RNA from FFPE Tissue Samples
CD504	RNAstorm™ RNA Isolation Kit
31030	DNA Gel Extraction Kit

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