

# Product Information

## AccuClear™ Ultra High Sensitivity dsDNA Quantitation Solution

Catalog Number: 31027-T, 31027

### Unit Size:

31027-T: 250 assays (200 uL microplate assay)

31027: 1000 assays (200 uL microplate assay)

### Kit Contents

Component	31027-T	31027
99977: AccuClear™ dye (100X in DMSO)	0.5 mL	2 X 1 mL
99978: AccuClear™ buffer	50 mL	200 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 Solution provides highly sensitive and accurate DNA quantitation across a broad range of DNA concentrations (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 microplate format. Unlike absorbance-based measurements, AccuClear™ dye is highly selective for double-stranded DNA over single stranded DNA or RNA (Figure 3).

The AccuClear™ 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 Quantitation Solution includes AccuClear™ dye and assay buffer. Biotium also offers the AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit (catalog no. 31028), which includes dye, buffer, and a set of pre-diluted calf thymus dsDNA standards. For large numbers of samples, Biotium offers the AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit with 1 DNA Standard (4000 assays, cat. no. 31029) which includes dye, 20X assay buffer, and a 25 ng/uL DNA standard stock solution that can be used to prepare a range of standards.

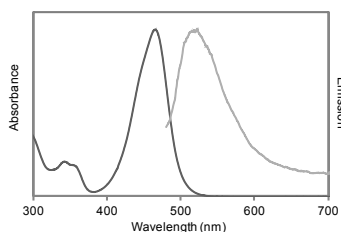


Figure 1. Absorbance and emission spectra of AccuClear™ dye bound to dsDNA.

### 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.
2. Prepare DNA standards in 1X TE buffer using the dsDNA of your choice. Prepare a 25 ng/uL stock solution of DNA. Determine the DNA concentration on the basis of absorbance at 260 nm in a cuvette with a 1 cm path length. An  $A_{260}$  value of 0.5 corresponds to a concentration of 25 ng/uL. Perform eight 3-fold serial dilutions of the 25 ng/uL DNA solution to obtain standards ranging between 25 ng/uL and 0.0038 ng/uL (3.8 pg/uL). Use 1X TE as the zero DNA standard. We recommend preparing the two lowest concentration standards fresh before each assay because these standards tend to decrease in concentration during storage, possibly due to adsorption of DNA to the tube. The higher concentration DNA standards can be stored at 4°C. For long term storage we recommend adding sodium azide to a final concentration of 2 mM.
3. 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.
4. 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 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.
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 into its own separate well containing working solution and mix well by pipetting up and down.
7. Pipette 10 uL of each unknown DNA into its own separate well containing working solution and mix well by pipetting up and down.
8. Incubate the microplate at room temperature for 5 minutes in the dark.
9. 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).

Continued next page

10. Generate a standard curve to determine the unknown DNA concentration (see Figure 2). Average the triplicate values for each 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 ( $y = \text{fluorescence}$  and  $x = \text{ng DNA per well}$ ). 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. Biotium also offers AccuClear™ dsDNA Quantitation Kit (catalog no. 31028), which includes a set of pre-diluted calf thymus dsDNA standards.

The linear range of the AccuClear™ assay extends from 0.03 ng to 250 ng. The standard curve can be extended to 300 ng with some loss of linearity. If the fluorescence of any of the unknown samples is higher than the linear range, further dilute the sample and add 10  $\mu\text{L}$  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™.

Table 1. Effect of common DNA contaminants on AccuClear™ assay signal

Compound	Initial concentration in DNA sample	Final concentration in assay (200 $\mu\text{L}$ )	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 $\mu\text{M}$	11%

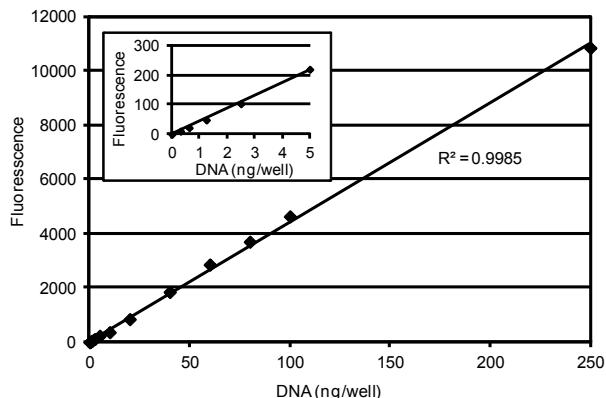


Figure 2. Linearity of AccuClear™ Ultra High Sensitivity dsDNA Quantitation Solution between 38 pg and 250 ng per well in microplate assay with excitation/emission at 468/507 nm. The inset shows the lower portion of the curve. Note: the graph shown above is for reference only. You must generate your own standard curve using your instrument to calculate the amount of DNA in your unknown samples.

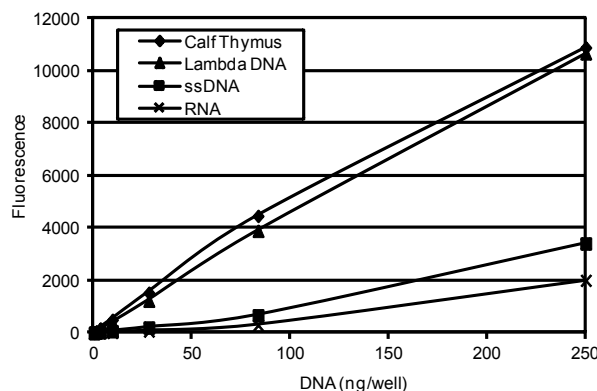


Figure 3. Selectivity of AccuClear™ Ultra High Sensitivity dsDNA Quantitation Solution for double-stranded DNA compared to single stranded DNA and single-stranded RNA.

### Related Products

Catalog number	Product
31028	AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit with 7 DNA Standards (1000 assays)
31029	AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit with 1 DNA Standard (4000 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](http://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.

AccuClear technology is covered by pending US patents. Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.

