

# Product Information

## AccuClear® Ultra High Sensitivity dsDNA Quantitation Solution

### Catalog Number and Kit Size

31027-T: 250 assays

31027: 1000 assays

### Kit Contents

Component	31027-T	31027
AccuClear® dye (100X in DMSO)	99977-T 0.5 mL	99977 2 X 1 mL
DNA Quantitation Buffer, 20X Concentrate	99979-3mL 3 mL	99979-10mL 10 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 Solution provides 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/μL to 25 ng/μL 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.

This AccuClear® Ultra High Sensitivity dsDNA Quantitation Solution includes just AccuClear® dye and assay buffer, requiring the user to obtain their own DNA standards. For more convenience, Biotium also offers the AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit (cat. no. 31028), which includes dye, buffer, and a set of pre-diluted calf thymus dsDNA standards. For large numbers of samples we offer a 2000 assay kit (cat. no. 31029) that includes dye, buffer, and a 25 ng/μL DNA standard stock solution that can be used to prepare a range of standards.

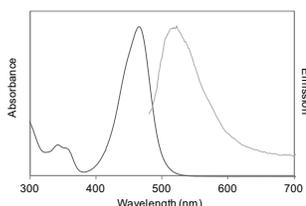


Figure 1. Absorbance and emission spectra of AccuClear® dye bound to dsDNA.

### Assay Protocol

The first time that you use this kit, dilute the 20X buffer concentrate to 1X by adding dH<sub>2</sub>O directly to the bottle containing the concentrated buffer. To 99979-3mL add 57 mL dH<sub>2</sub>O; to 99979-10mL add 190 mL dH<sub>2</sub>O. 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.

**Note:** If you are using an older version of this kit that came with 1X buffer (component 99978) instead of 20X concentrate, you can disregard the buffer dilution instructions.

- 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.
- Prepare DNA standards according to the DNA Standard Preparation instructions on page 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.
- On the day of the assay, prepare 200 μL 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 μL 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.
- For each sample to be tested, pipette 200 μL 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.
- Add 10 μL 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.
- 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.
- 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).
- 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.

## DNA Standard Preparation

Prepare DNA standards in 1X DNA Quantitation Buffer or 1X TE 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. Prepare the set of standards. We suggest making 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). 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.

## 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. Biotium also offers AccuClear® dsDNA Quantitation Kit (cat. no. 31028), which includes a set of pre-diluted calf thymus dsDNA standards. A 25 ng/uL calf thymus dsDNA standard is also available separately (cat. no. 31029C).

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.

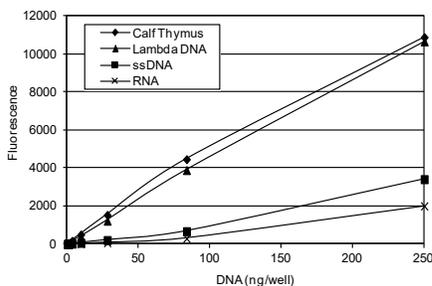


Figure 2. Selectivity of AccuClear® dsDNA quantitation assay for double-stranded DNA compared to single stranded DNA and single-stranded RNA.

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.

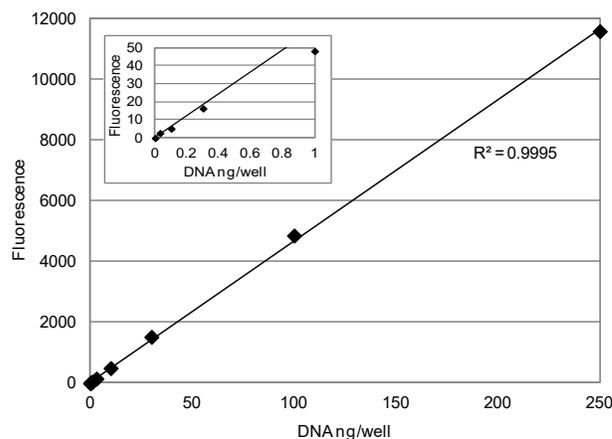


Figure 3. Linearity of AccuClear® Ultra High Sensitivity dsDNA Quantitation Solution between 30 pg and 250 ng per well in microplate assay with excitation/emission at 468/507 nm. The inset shows the lower portion of the curve. Note: the graph shown above is for reference only. You must generate your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

## Related Products

Catalog number	Product
31029C	AccuClear® dsDNA Standard, 25 ng/uL
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 (2000 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	RNAstorn™ Kit for Isolation of RNA from FFPE Tissue Samples
CD202	DNASTorn™ Kit for Isolation of RNA from FFPE Tissue Samples
CD504	RNAstorn™ RNA Isolation Kit
31030	DNA Gel Extraction Kit

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. AccuBlue and AccuClear are covered by granted and/or pending US and/or international patents.

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