

Product Information

AccuGreen™ High Sensitivity dsDNA Quantitation Solution

For use with handheld fluorometers such as Qubit®

Kit Contents

Component	31068-T 100 assays	31068 500 assays
AccuGreen™ Dye, 200X	99817-T 250 uL	99817 1.25 mL
DNA Quantitation Buffer, 20X Concentrate	99979-3mL 3 mL	99979-12.5mL 12.5 mL

Reagents to be supplied by user

0.5 mL clear PCR tubes
10 ng/uL DNA standard

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. The dye color may change when stored at 4°C, but this will not affect performance. AccuGreen™ dye is stable for storage at room temperature for up to 6 months. AccuGreen™ dye is a potentially harmful chemical. Exercise universal laboratory safety precautions when handling the dye.

Spectral Properties

Ex/Em: 509/530 nm (bound to dsDNA). See Figure 1 for spectra.

Product Description

The AccuGreen™ High Sensitivity dsDNA Quantitation Solution is designed for use with handheld fluorometers such as the Qubit® fluorometers from Thermo Fisher. Unlike absorbance-based measurements, AccuGreen™ Dye is highly selective for double-stranded DNA over single-stranded DNA or RNA.

The linear range of the AccuGreen™ assay is 0.1 to 100 ng of DNA per tube. DNA samples with concentrations between 5 pg/uL and 100 ng/uL may be quantified using sample volumes between 1 and 20 uL (for example, 1 uL of 100 ng/uL is 100 ng total, and 20 uL of 5 pg/uL is 0.1 ng total, which will both fall within the linear range of the assay). If you use the most common sample volume of 10 uL, the starting sample concentration should be 10 pg/uL to 10 ng/uL.

The AccuGreen solutions provide enough reagents to quantify approximately 100 samples (31068-T) or 500 samples (31068), plus the two standards. There are enough reagents for 250 reactions (31068-T) or 1,250 reactions (31068) including the standards.

Biotium also offers the AccuGreen™ High Sensitivity dsDNA Quantitation Kit (31066), which includes dye, buffer, and a calf thymus dsDNA standard.

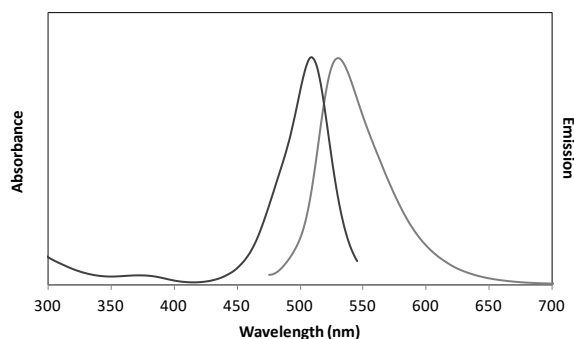


Figure 1. Absorbance and emission spectra of AccuGreen™ Dye bound to dsDNA.

Protocol for reading the AccuGreen™ assay on the Qubit® Fluorometer

The first time that you use this kit, dilute the 20X buffer concentrate to 1X by adding dH₂O directly to the bottle containing the concentrated buffer. To 99979-3mL add 57 mL dH₂O; to 99979-12.5mL add 237.5 mL dH₂O. 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.

This protocol describes how to measure AccuGreen™ fluorescence on a Qubit® 3.0 Fluorometer using the pre-programmed dsDNA High Sensitivity program. Instructions may vary for older Qubit® models.

Note: The linear range for this assay on the Qubit® 3.0 is 100 pg-100 ng DNA in the assay tube. However, samples even slightly below 100 pg will return the error message "Out of Range". Therefore for best results use more than 100 pg DNA per assay.

1. Prepare or obtain a 10 ng/uL DNA standard in 1X TE buffer using the dsDNA of your choice (see Considerations for Data Analysis, next page). The DNA concentration can be determined on the basis of absorbance at 260 nm in a cuvette with a 1 cm path length. An A₂₆₀ value of 0.2 corresponds to a concentration of 10 ng/uL. Use 1X DNA Quantitation Buffer as the zero DNA standard. The DNA standard can be stored at 4°C. For long term storage we recommend adding sodium azide to a final concentration of 2 mM.
2. Warm all components to room temperature before use. AccuGreen™ 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.
3. Prepare the 1X working solution by diluting 200X AccuGreen™ Dye 1:200 in 1X DNA Quantitation Buffer. You will need 200 uL of working solution for each standard and sample to be tested. For example, to quantify three DNA samples, you will need 1 mL of working solution (2 standards and 3 samples).
4. For each sample and standard, pipette 190 uL of the working solution into a clear 0.5 mL PCR tube (if using the Qubit® fluorometer).
5. Into one tube, pipet 10 uL of AccuGreen™ Buffer (Standard 1).
6. Into a second tube, pipet 10 uL of the 10 ng/uL DNA prepared in Step 1 (Standard 2).
7. Pipette 1-20 uL of each DNA sample to be quantified into its own tube.
8. Gently vortex or invert the tubes several times to mix, and then incubate the tubes at room temperature for at least 2 minutes.
9. Turn on the Qubit® 3.0 instrument. On the home screen select dsDNA. Choose the High Sensitivity assay.
10. Follow the prompts on the screen, and first read the AccuGreen™ Standard 1 and then the AccuGreen™ Standard 2. The program will use these values to quantify your unknown samples.
11. One at a time, measure each of your samples.
12. The data can be recorded manually or exported as a csv file.

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. 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 effects of common DNA contaminants such as salts, solvents, detergents and protein on the AccuGreen™ assay are listed in Table 1. The same contaminants were tested in the Qubit® HS DNA assay, and found to give essentially identical results.

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

Compound	Initial concentration in DNA sample	Final concentration in assay (200 uL)	Signal change (negative value indicates decrease)
Sodium Chloride	1 M	50 mM	-13%
Magnesium Chloride	100 mM	5 mM	-21%
Sodium Acetate	600 mM	30 mM	-16%
Ammonium Acetate	1 M	50 mM	-14%
Ethanol	20%	1%	+9%
Phenol	2%	0.10%	+4%
SDS	0.2%	0.01%	-89%
SDS	0.02%	0.001%	-8%
Triton™ X-100	2%	0.1%	+1%
Triton™ X-100	0.02%	0.001%	+4%
Tween® 20	0.1%	0.005%	+4%
CTAB	0.01%	0.0005%	-96%
BSA	2 mg/mL	0.1 mg/mL	no change
dNTPs	2 mM	100 uM	+2%

Related Products

Catalog number	Product
31066	AccuGreen™ High Sensitivity dsDNA Quantitation Kit
31069	AccuGreen™ Broad Range dsDNA Quantitation Kit
31070	AccuGreen™ Broad Range dsDNA Quantitation Solution
31060	AccuBlue® NextGen dsDNA Quantitation Kit
31028	AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit with 7 DNA Standards
31007	AccuBlue® Broad Range dsDNA Quantitation Kit with 9 DNA Standards
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
31041	Forget-Me-Not™ EvaGreen® qPCR Master Mix (2-Color Tracking)
31045-T	Forget-Me-Not™ EvaGreen® qPCR Master Mix (low ROX)
31046-T	Forget-Me-Not™ EvaGreen® qPCR Master Mix (high ROX)
31043	Forget-Me-Not™ Universal Probe Master Mix
22020	10X Phosphate-Buffered Saline (PBS)
41024-4L	Water, Ultrapure Molecular Biology Grade
31030	DNA Gel Extraction Kit
CD504	RNAstorm™ RNA Isolation Kit
CD501	RNAstorm™ Kit for Isolation of RNA from FFPE Tissue Samples
CD502	DNASTORM™ Kit for Isolation of RNA from FFPE Tissue Samples

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.

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