

Product Information

AccuGreen™ High Sensitivity dsDNA Quantitation Kit

For use with handheld fluorometers such as the Qubit®

Kit Contents

Component	31066-T 100 assays	31066 500 assays
AccuGreen™ Dye, 200X	250 uL 99817-T	1.25 mL 99817
AccuGreen™ Buffer, 1X	50 mL 99818-T	250 mL 99818
AccuGreen™ Standard 1 (0 ng/uL)	1 mL 99819-T	5 mL 99819
AccuGreen™ Standard 2 (10 ng/uL)	1 mL 99820-T	5 mL 99820

Reagents to be supplied by user

0.5 mL clear PCR tubes

Storage and Handling

It is recommended that the Dye and Buffer components of the kit be stored at room temperature, although they can also be stored at 4°C if desired. The DNA Standards should be stored at 4°C. Protect Dye from light. The dye color may change when stored at 4°C, but this will not affect performance. The kit is stable for at least 12 months from date of receipt when stored as recommended. AccuGreen™ 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: 509/530 nm (bound to dsDNA). See Figure 1 for spectra.

Product Description

The AccuGreen™ High Sensitivity dsDNA Quantitation Kit is designed for use with handheld fluorometers such as the Qubit® fluorometer from Thermo Fisher. The AccuGreen™ assay is linear between 0.1 and 100 ng of dsDNA per assay, which corresponds to sample concentrations of 10 pg/uL to 10 ng/uL. Unlike absorbance-based measurements, AccuGreen™ Dye is highly selective for double-stranded DNA over single-stranded DNA or RNA.

The AccuGreen kits provide enough reagents to quantify approximately 100 samples (31066-T) or 500 samples (31066), plus the two standards. There are enough reagents for 250 reactions (31066-T) or 1,250 reactions (31066) including the standards.

Biotium also offers the AccuGreen™ High Sensitivity dsDNA Quantitation Solution (catalog no. 31068). While the AccuGreen™ Kit comes with a calf thymus DNA standard, the AccuGreen™ Solution does not provide a DNA standard. It is intended for those who wish to provide their own standard.

For high-sensitivity DNA quantitation using a fluorescent plate reader, we recommend using our AccuClear® or AccuBlue® dsDNA Quantitation Kits. The AccuClear® assay (31028) combines excellent sensitivity and the broadest linear range. The AccuBlue® NextGen assay (31060) provides unprecedented sensitivity and accuracy.

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

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 (corresponding to sample concentrations of 10 pg/uL-10 ng/uL). However, samples even slightly below 10 pg/uL will return the error message "Out of Range". Therefore for best results you should use samples above 10 pg/uL.

1. 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.
2. Prepare the 1X working solution by diluting 200X AccuGreen™ Dye 1:200 in AccuGreen™ 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).
3. 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).
4. Into one tube, pipet 10 uL of AccuGreen™ Standard 1 (0 ng/uL).
5. Into a second tube, pipet 10 uL of AccuGreen™ Standard 2 (10 ng/uL).
6. Pipette 10 uL of each DNA sample to be quantified into its own tube.
7. Incubate the tubes at room temperature for at least 2 minutes.
8. Turn on the Qubit™ 3.0 instrument. On the home screen select dsDNA. Choose the High Sensitivity assay.
9. 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.
10. One at a time, measure each of your samples.
11. The data can be recorded manually or exported as a csv file.

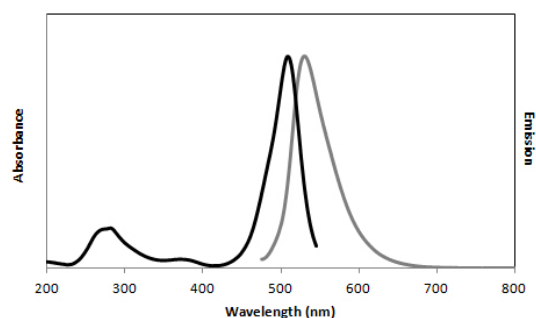


Figure 1. Absorbance and emission spectra of AccuGreen™ Dye bound to dsDNA.

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
31068	AccuGreen™ High Sensitivity dsDNA Quantitation Solution
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
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
CD501	RNAstom™ Kit for Isolation of RNA from FFPE Tissue Samples
CD502	DNASTom™ 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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