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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

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Product Information

AccuBlue® Broad Range dsDNA Quantitation Solution

Catalog Number and Kit Size

31009-T: 200 assays
31009: 1500 assays

Kit Contents

Component	31009-T	31009
AccuBlue® Broad Range Buffer	99975 50 mL	99837 300 mL
AccuBlue® Broad Range Dye (100X in DMSO)	99974 1 mL	99974 3 x 1 mL
AccuBlue® Broad Range Enhancer (100X in water)	99943 1 mL	99943 3 x 1 mL

Storage and Handling

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

Spectral Properties

Ex/Em 350/460nm (in the presence of dsDNA). See Figure 1 for spectra.

Product Description

The AccuBlue® Broad Range Quantitation Solution provides sensitive and accurate DNA quantitation over a broad range of DNA concentrations. Unlike absorbance-based measurements, the assay is selective for dsDNA over ssDNA or RNA (Figure 2). This assay is linear between 2 ng and 2000 ng of dsDNA per assay (0.2 ng/uL to 200 ng/uL sample concentration) in 96-well microplate format (Figure 3).

The AccuBlue® Broad Range dsDNA Quantitation Solution is designed for use with 96-well plates and fluorescence microplate readers equipped with excitation and emission filters for detecting blue fluorescence. The AccuBlue® Broad Range Quantitation assay offers the advantages of a wide dynamic range and high sensitivity over other traditional methods of DNA quantitation.

For added convenience, we also offer a kit with a set of DNA standards (cat. no. 31007).

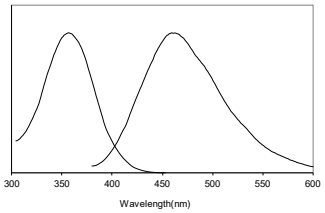

Figure 1: Excitation and emission spectra for AccuBlue® Broad Range dsDNA quantitation reagent in the presence of excess dsDNA.

Assay Protocol

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. Prepare DNA standards according to the DNA Standard Preparation instructions on page 2.

3. Warm all components to room temperature before use. AccuBlue® 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.

4. On the day of the assay, prepare 200 uL of working solution for each sample to be tested. Dilute both the Dye and the Enhancer 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 and 200 uL of Enhancer with 20 mL Buffer to prepare enough working solution for an entire 96 well plate. Volumes can be scaled as required. Working solution should be used within an hour after preparation for best results, but it can be stored and used up to 24 hours later, with only minor loss of accuracy. During storage dye precipitation may occur, but can be resuspended by vortexing.

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 TE as the zero standard.

7. Incubate the microplate at room temperature for 10 minutes in the dark. The assay plate should be read within 1 hour for best results, but can be stored and read up to 24 hours after preparation with only minor loss of accuracy.

8. Measure fluorescence using a microplate reader with 350 nm excitation/460 nm emission.

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.

DNA Standard Preparation

Prepare DNA standards in 1X TE buffer using the dsDNA of your choice. Prepare a 200 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} of 1 corresponds to a 50 ng/uL dsDNA solution. Because most spectrophotometers saturate around an absorbance of 2, dilute the 200 ng/uL stock 1:10 and take the OD_{260} of the diluted stock solution. An A_{260} of 0.4 corresponds to a concentration of 20 ng/uL. Factor in the dilution to obtain the concentration of the original stock solution.

We suggest making six 2-fold serial dilutions of the 200 ng/uL DNA solution to obtain standards ranging between 200 ng/uL and 3.125 ng/uL. For long term storage, we recommend adding sodium azide to a final concentration of 2 mM and storing DNA standards at 4°C.

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 AccuBlue® Broad Range dsDNA Quantitation Kit is available with calf thymus DNA standards (cat. no. 31007).

Fluorescence quantitation by the AccuBlue® Broad Range assay is linear from 2 – 2000 ng dsDNA. If lower end standards are desired, you can further dilute any of the standards with 1X TE to a concentration of 0.2 ng/uL, and add 10 uL/well to obtain a 2 ng/well standard.

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) pipette accuracy, and (5) microplate manufacturers.

The effects of common DNA contaminants such as salts, solvents, detergents and protein on the AccuBlue® Broad Range assay are listed in Table 2. Please also see our AccuBlue® High Sensitivity and AccuClear® Ultra High Sensitivity dsDNA Quantitation Assays (related products), which have different tolerances for certain contaminants compared to AccuBlue® Broad Range.contaminants compared to AccuBlue® Broad Range.

Table 2. Effect of common DNA contaminants on AccuBlue® Broad Range assay

Compound	Initial concentration in DNA sample	Final concentration in assay (200 uL)	Decrease in Signal
Sodium Chloride	500 mM	25 mM	-5%
Magnesium Chloride	100 mM	5 mM	35%
Sodium Acetate	600 mM	30 mM	50%
Ethanol	20%	1%	4%
Phenol	2%	0.10%	3%
SDS	0.2%	0.01%	32%
SDS	0.02%	0.001%	5%
Triton X-100	0.2%	0.01%	8%
Triton X-100	0.02%	0.001%	-5%
Tween-20	0.1%	0.005%	20%
CTAB*	0.01%	0.0005%	64%
dNTPs	2 mM	100 uM	-1%
BSA**	0.8 mg/mL	0.05 mg/mL	27%

* Average change for 250 ng to 2000 ng samples. Complete loss of signal is seen below 250 ng.

** Average change for 20 ng to 2000 ng samples. Not compatible with quantitation below 20 ng due to increased background at low DNA concentrations

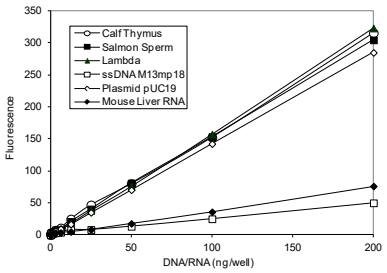

Figure 2. Relative fluorescence intensities of different nucleic acids using the AccuBlue® Broad Range dsDNA Quantitation kit.

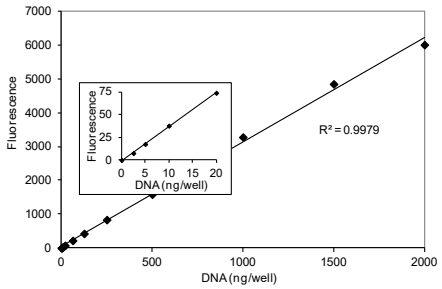

Figure 3: Standard curve of calf thymus DNA assayed using AccuBlue® Broad Range Kit and read on a microplate reader (Ex/Em 350/460). Inset shows the lower end of the titration.

Related Products

Catalog number	Product
31069	AccuGreen™ Broad Range dsDNA Quantitation Kit (for Qubit®)
31007	AccuBlue® Broad Range dsDNA Quantitation Kit
31060	AccuBlue® NextGen dsDNA Quantitation Kit
31066	AccuGreen™ High Sensitivity dsDNA Quantitation Kit (for Qubit)
31028	AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit
31006	AccuBlue® High Sensitivity dsDNA Quantitation Kit with 8 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
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
CD504	RNAstorm™ RNA Isolation Kit
31030	DNA Gel Extraction Kit

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.