Product Information

AccuBlue™ NextGen dsDNA Quantitation Solution

Catalog Number:
31061-T AccuBlue™ NextGen dsDNA Quantitation Solution, trial size
31061 AccuBlue™ NextGen dsDNA Quantitation Solution

Kit Contents

Component	31061-T 200 assays	31061 1000 assays
AccuBlue™ NextGen Dye, 400X	1 X 100 uL 99808-T	1 x 500 uL 99808
AccuBlue™ NextGen Enhancer, 100X	1 x 1 mL 99809	2 x 1 mL 99809
AccuBlue™ NextGen Buffer, 1X	1 x 50 mL 99810-T	1 x 250 mL 99810-250mL

Storage and Handling
Store kit at 4°C. Protect AccuBlue™ NextGen Dye from light. The kit is stable for at least 12 months from date of receipt when stored as recommended. AccuBlue™ NextGen 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
The AccuBlue™ NextGen dsDNA Quantitation Solution provides unprecedented accuracy and sensitivity to low amounts of DNA (ref. 1). It is ideal for use in quantifying DNA from low-concentration or precious samples, and for quantifying DNA for use in sensitive applications such as Next-Gen Sequencing (NGS) or digital PCR. The AccuBlue™ NextGen assay is linear between 5 pg and 3 ng of dsDNA per assay (see Figure 2), which corresponds to sample concentrations of 0.5 pg/uL to 300 pg/uL in the 96-well microplate format (depending on the sensitivity of the plate reader, see Table 1). Unlike absorbance-based measurements, AccuBlue™ NextGen Dye is highly selective for double-stranded DNA over single stranded DNA or RNA.

The AccuBlue™ NextGen dsDNA Quantitation assay is designed for use with fluorescence microplate readers equipped with excitation and emission filters for detecting green fluorescence. The unique spectral properties of AccuBlue™ NextGen Dye make it especially well-suited for use with instruments with blue LED excitation sources.

We also offer AccuBlue™ NextGen dsDNA Quantitation Kit, which includes a calf thymus dsDNA standard (see related products).

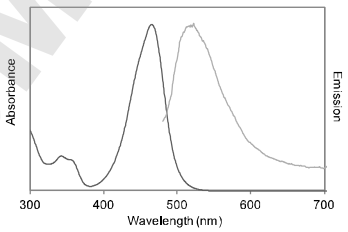

Figure 1. Absorbance and emission spectra of AccuBlue™ NextGen Dye bound to dsDNA.

Assay Considerations

1. Because the AccuBlue™ NextGen dsDNA Quantitation assay is so sensitive, results will vary depending on several factors including: microplate reader sensitivity and accuracy, pipeting accuracy, and assay volume. Use properly calibrated pipettes and DNase-free pipette tips, tubes and plates for best accuracy.

2. DNA in low concentrations such as in this assay range (0.5-300 pg/uL) is susceptible to loss due to adsorption and/or denaturation when stored in polypropylene tubes (ref. 2). For best results, dilute samples should be quantified shortly before they will be used. Using low-binding tubes may also help prevent adsorption.

3. It is recommended to test each DNA standard and each unknown sample in triplicate. Because the assay sensitivity is highly dependent on the accuracy and sensitivity of the plate reader, we recommend performing an inital standard curve to determine the linear range supported by your plate reader (results for the plate readers we have tested are shown in Table 1 as a guideline). See Considerations for Data Analysis (next page) for more information on DNA standards.

Assay Protocol

1. Warm all components to room temperature before use. AccuBlue™ NextGen 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.

2. Prepare a DNA standard in 1X TE with the dsDNA of your choice. Prepare a 10 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 pathlength. An A_{260} of 0.2 corresponds to a 10 ng/uL dsDNA solution. The 10 ng/uL DNA stock solution can be stored at 4°C for at least 6 months with the addition of sodium azide as a preservative at 2 mM final concentration.

3. Use the 10 ng/uL DNA Standard to create the standards for the standard curve by diluting in 1X AccuBlue™ NextGen Buffer as shown below. If your reader is sensitive enough to detect DNA amounts below 5 pg, you can follow the instructions for preparing 0.25 and/or 0.1 pg/uL standards in Considerations for Data Analysis (next page). Volumes may be scaled as necessary. **The DNA dilutions should be prepared fresh on the day of assay, and any leftover diluted standards should be discarded if they won't be used that day.** See Assay Considerations point 2 above.

Standard	Concentration	DNA	1X TE buffer
A	300 pg/uL	12 uL of 10 ng/uL DNA Standard	388 uL
B	50 pg/uL	10 uL of A	50 uL
C	10 pg/uL	12 uL of B	48 uL
D	2 pg/uL	12 uL of C	48 uL
E	0.5 pg/uL	15 uL of D	45 uL
F	0 ng/uL	–	60 uL

4. Prepare working solution. You will need 200 uL of working solution for each standard and sample to be tested. Working solution can be safely stored, protected from light, for 24 hours at ambient temperature or 6 months at 4°C.

5. Prepare working solution by diluting AccuBlue™ NextGen Dye at a ratio of 1:400 and AccuBlue™ NextGen Enhancer at a ratio of 1:100, in AccuBlue™

NextGen Buffer. For example, to test 6 standards and 4 samples in triplicate, you would add 15 uL Dye and 60 uL Enhancer to 6 mL Buffer. Mix well by vortexing or shaking to ensure that Dye and Enhancer are fully dispersed.

6. For each sample to be tested, pipette 200 uL of the working solution per well of a black flat-bottom 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.

7. Add 10 uL of each dsDNA standard into its own separate well containing working solution and mix well by pipetting up and down.

8. Pipette 10 uL of each unknown DNA into its own separate well containing working solution and mix well by pipetting up and down.

9. Incubate the microplate at room temperature for at least 5 minutes in the dark. The reaction plate can be stored up to 24 hours at ambient temperature before reading.

10. Measure fluorescence using a microplate reader set to 468 nm excitation/507 nm emission maxima or other similar filter combination for detecting green fluorescence.

11. Generate a standard curve to determine the unknown DNA concentration (see Figure 2). Average the triplicate values for each sample and subtract the average 0 ng DNA value from each data point. Plot the fluorescence values for the DNA standards on the y-axis and pg/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 = pg 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. 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 stated linear range of the AccuBlue™ NextGen dsDNA Quantitation assay extends from 5 pg to 3 ng, when following the above protocol. We have observed accuracy down to 1 pg or lower, depending on the microplate reader and assay volume (Table 1). If lower concentration standards are desired, you can prepare 0.25 pg/uL and/or 0.1 pg/uL standards. For 0.25 pg/uL, combine 5 uL of the 2 pg/uL standard with 35 uL buffer; for 0.1 pg/uL, combine 10 uL of the 0.5 pg/uL standard with 40 uL of buffer. Use 10 uL of these standards in the assay to obtain 2.5 pg and 1 pg data points.

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 (gain) settings, (4) pipetting accuracy, and (5) microplate manufacturer.

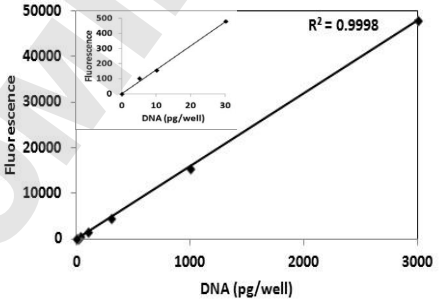

Figure 2. Linearity of AccuBlue™ NextGen dsDNA quantitation assay between 5 and 3000 pg per well in a 96-well microplate assay with excitation/emission at 468/507 nm. The inset shows the lower portion of the curve.

Table 1

reader	assay format (assay volume)	Lowest DNA detected
BMG CLARIOStar®	96-well (200 uL)	1 pg
	384-well (50 uL)	0.6 pg
Biotek Synergy™ H1m	96-well (200 uL)	5 pg
Spectramax® Gemini XS	96-well (200 uL)	5 pg
Spectramax® i3	384-well (50 uL)	1.25 pg

Table 2

Compound	Initial concentration in DNA sample	Final concentration in assay (200 uL)	Decrease in Signal
Sodium Chloride	500 mM	25 mM	7%
Magnesium Chloride	100 mM	5 mM	30%
Sodium Acetate	600 mM	30 mM	11%
Ethanol	20%	1%	8%
Phenol	2%	0.10%	10%
SDS	0.2%	0.01%	87%
SDS	0.02%	0.001%	13%
Triton X-100	0.2%	0.01%	18%
Triton X-100	0.02%	0.001%	8%
Tween-20	0.1%	0.005%	8%
CTAB*	0.01%	0.0005%	100%
dNTPs	2 mM	100 uM	1%
BSA**	0.8 mg/mL	0.04 mg/mL	19%

* CTAB generally inhibits fluorescence, but at 5 pg the background is increased.
** BSA is not compatible with quantitation below 500 pg due to increased background at low DNA concentrations

References

1. B. Bruijns, R. Tiggelaar, H. Gardeniers. Fluorescent cyanine dyes for the quantification of low amounts of dsDNA. Analytical Biochemistry (2016), doi: 10.1016/j.ab.2016.07.022.

2. Claire Gaillard, François Strauss. Avoiding adsorption of DNA to polypropylene tubes and denaturation of short DNA fragments. Technical Tips Online. Volume 3, Issue 1, pp. 63-65 (1998).

Related Products

Catalog number	Product
31060	AccuBlue™ NextGen dsDNA Quantitation Kit
31028	AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit with 7 DNA Standards
31006	AccuBlue™ High Sensitivity dsDNA Quantitation Kit
31007	AccuBlue™ Broad Range dsDNA Quantitation Kit
31066	AccuGreen™ High Sensitivity dsDNA Quantitation Kit (for Qubit)
41003	GelRed™ Nucleic Acid Gel Stain, 10,000X in water
31041-T	Forget-Me-Not™ qPCR Master Mix, trial size
31043-T	Forget-Me-Not™ Universal Probe Master Mix, trial size

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.