

Product Information

AccuBlue® NextGen dsDNA Quantitation Solution

Catalog Number and Kit Size

31061-T: 200 assays

31061: 1000 assays

Kit Contents

Component	31061-T	31061
AccuBlue® NextGen Dye, 400X	99808-T 100 uL	99808 500 uL
AccuBlue® NextGen Enhancer, 100X	99809 1 mL	99809 2 x 1 mL
DNA Quantitation Buffer, 20X Concentrate	99979-3mL 3 mL	99979-12.5mL 12.5 mL

*Note: The buffer in this kit was changed from 1X to 20X.

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).

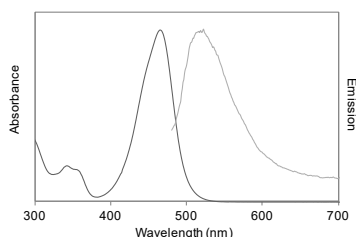


Figure 1. Absorbance and emission spectra of AccuBlue® NextGen Dye bound to dsDNA.

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 black 96-well microplates (to minimize fluorescence bleed-through between wells), properly calibrated pipettes and DNase-free plasticware 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 initial 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

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.

Note: If you are using an older version of this kit that came with 1X buffer (component 99810) instead of 20X concentrate, you can disregard the buffer dilution instructions.

1. Warm all components to room temperature before use. AccuBlue® NextGen 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.
2. Prepare a 10 ng/uL DNA standard in 1X TE with the dsDNA of your choice. Determine the DNA concentration on the basis of absorbance at 260 nm in a cuvette with a 1 cm pathlength. An A₂₆₀ 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 High Sensitivity DNA Standard to create the standards for the standard curve by diluting in 1X DNA Quantitation 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

- Prepare 200 μ L 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.
- 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 1X DNA Quantitation Buffer. For example, to test 6 standards and 4 samples in triplicate, you would add 15 μ L Dye and 60 μ L Enhancer to 6 mL Buffer. Mix well by vortexing or shaking to ensure that Dye and Enhancer are fully dispersed.
- For each sample to be tested, pipette 200 μ L 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 μ L of each dsDNA standard and unknown into its own separate well containing working solution and mix well by pipeting up and down. Use 1X DNA Quantitation Buffer or 1X TE as the zero standard.
- 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.
- Measure fluorescence using a microplate reader set to 468 nm excitation/507 nm emission maxima or other similar filter combination for detecting green fluorescence.
- To determine the unknown DNA concentration generate a standard curve (see Figure 2). 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.

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. Biotium also offers AccuBlue® NextGen dsDNA Quantitation Kit (cat. no. 31060), which includes a calf thymus dsDNA standard. A 10 ng/ μ L calf thymus dsDNA standard is also available separately (cat. no. 99820-T).

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/ μ L and/or 0.1 pg/ μ L standards. For 0.25 pg/ μ L, combine 5 μ L of the 2 pg/ μ L standard with 35 μ L buffer; for 0.1 pg/ μ L, combine 10 μ L of the 0.5 pg/ μ L standard with 40 μ L of buffer. Use 10 μ L of these standards in the assay to obtain 2.5 pg and 1 pg data points. It is recommended to prepare the standards on the same day as the assay is performed.

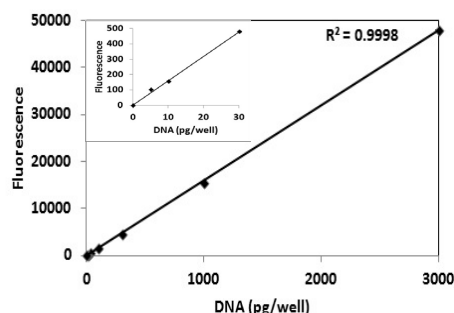


Figure 2. Linearity of AccuBlue® NextGen dsDNA quantitation assay between 5 and 3000 pg per well in a 96-well microplate assay with excitation/emission at 468/507 nm. The inset shows the lower portion of the curve.

Table 1

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

Table 2

Compound	Initial concentration in DNA sample	Final concentration in assay (200 μ L)	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 μ M	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

- B. Buijns, 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.
- 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
99820-T	10 ng/ μ L Calf Thymus DNA Standard
31060	AccuBlue® NextGen dsDNA Quantitation Kit
31028	AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit with 7 DNA Standards
31073	AccuBlue® Broad Range RNA Quantitation Kit
31066	AccuGreen™ High Sensitivity dsDNA Quantitation Kit (for Qubit®)
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in water
31041	Forget-Me-Not™ EvaGreen® qPCR Master Mix, 2-Color Tracking
31043	Forget-Me-Not™ Universal Probe Master Mix

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