Product Information

AccuBlue® Broad Range RNA Quantitation Kit

Catalog Number: 31073, 31073-T (Trial Size)

Kit Contents

Component	31073-T 200 assays	31073 1000 assays
RNA Broad Range Dye, 200X	99848-T 1 x 200 uL	99848 1 x 1 mL
RNA Broad Range Buffer	99849-T 1 x 50 mL	99849 1 x 250 mL
RNA Broad Range Standard, 100 ng/uL	99850 1 x 300 uL	99850 5 x 300 uL
RNA Dilution Buffer	99851-T 1 x 1 mL	99851 1 x 5 mL

Storage and Handling

Dye and buffer components may be stored at 4°C. The RNA standard should be stored at -20°C or below. Storage at -80°C will extend the shelf life of the RNA standard. Protect RNA Broad Range Dye from light. The kit is stable for at least 6 months from date of receipt when stored as recommended. RNA Broad Range 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: 650/670 nm (when bound to RNA).

Product Description

The AccuBlue® Broad Range RNA Quantitation Kit is a fluorescence-based assay designed to quantify purified RNA samples. The assay is linear between 5 and 1000 ng of RNA per 200 uL assay well (see Figure 2), which corresponds to sample concentrations of 0.5 to 100 ng/uL. It is ideal for use in quantifying RNA for sensitive applications such as Next-Gen Sequencing (NGS) or reverse transcription PCR (RT-PCR). Unlike absorbance-based measurements, RNA Broad Range Dye is highly selective for RNA over double-stranded DNA and can tolerate an equimolar amount of dsDNA in the sample without significant effect on RNA quantitation (Figure 3). Purified RNA samples are still recommended.

The AccuBlue® Broad Range RNA Quantitation assay is designed for use with fluorescence microplate readers equipped with excitation and emission filters for far-red fluorescence. It is also optimized for use in the Qubit® from Thermo Fisher, using the pre-programmed RNA Broad Range program (protocol on p. 2).

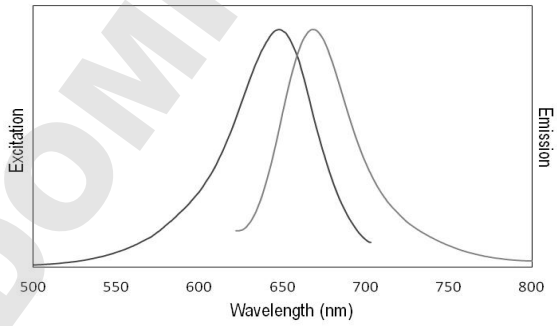

Figure 1. Excitation and emission spectra of RNA Broad Range Dye bound to RNA.

Assay Considerations

- The RNA Standard used in this kit is made from total mammalian cell RNA. When quantifying RNA from non-mammalian sources, it may be prefereable to use an RNA standard similar in size or source to your unknown samples.
- When using this kit, precautions should be taken to avoid contamination with RNases. RNase contamination of the RNA Standard or other kit components may result in errors with the quantitation. We suggest using nuclease-free tubes and filter tips.
- It is recommended to test each RNA standard and each unknown sample in triplicate for best accuracy.

Microplate Assay Protocol

1. Warm all components to room temperature before use. RNA Broad Range Dye is provided in DMSO, which may freeze during storage at 4°C.
2. Prepare 200 uL of working solution for each sample to be tested. Dilute the RNA Broad Range Dye in RNA Broad Range Buffer at a ratio of 1:200 in a plastic container and mix well by vortexing or shaking. For example, mix 100 uL of dye with 20 mL buffer to prepare enough working solution for an entire 96 well plate. Volumes can be scaled as required.
3. Prepare a set of RNA standards by diluting the 100 ng/uL RNA Broad Range Standard in RNA Dilution Buffer as shown in Table 1. The amounts in Table 1 are enough to perform one standard curve in triplicate. Volumes may be scaled as necessary.

Table 1. Preparation of RNA standards

Standard	Volume of RNA	Volume of RNA Dilution Buffer (uL)
100 ng/uL	Undiluted stock RNA	0 uL
50 ng/uL	20 uL of 100 ng/uL	20 uL
25 ng/uL	10 uL of 100 ng/uL	30 uL
10 ng/uL	5 uL of 100 ng/uL	45 uL
2 ng/uL	10 uL of 10 ng/uL	40 uL
0.5 ng/uL	10 uL of 2 ng/uL	30 uL
0 ng/uL	0 uL	100 uL

4. Pipet 200 uL of the working solution into each well of a black 96-well plate. Add 10 uL of RNA standard or RNA sample into each well, mixing by pipetting up and down. Test each standard and sample in triplicate wells for best accuracy.
5. Incubate the plate for 5-10 minutes in the dark.
6. Measure fluorescence using a microplate reader to set to 630 nm excitation/670 nm emission maxima or a filter combination with similar excitation/emission.
7. Generate a standard curve to determine the unknown RNA concentration. Average the triplicate values for each sample and subtract the average 0 ng value from each data point. Plot the fluorescence values for the RNA standards on the y-axis and total ng RNA/well 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 RNA in each well (y = fluorescence and x = ng RNA per well).

Qubit® Assay Protocol

Materials to be supplied by user

0.5 mL clear PCR tubes

This protocol describes how to measure RNA concentration on a Qubit® 3.0 Fluorometer using the pre-programmed RNA Broad Range program. Instructions may vary for older Qubit® models.

Note: The linear range for this assay on the Qubit® 3.0 is 5-1000 ng RNA in the assay tube (corresponding to sample concentrations of 0.5-100 ng/uL). However, samples even slightly below 0.5 ng/uL will return the error message "Out of Range". Therefore for best results you should use samples above 0.5 ng/uL.

1. Warm all components to room temperature before use. RNA Broad Range Dye is provided in DMSO, which may freeze during storage at 4°C.
2. Prepare 200 uL of working solution for each sample to be tested. Dilute the RNA Broad Range Dye in RNA Broad Range Buffer at a ratio of 1:200 in a plastic container and mix well by vortexing or shaking. For example, combine 10 uL of Dye with 2 mL Broad Range Buffer to prepare enough working solution for 10 tubes. Volumes can be scaled as required.
3. For each sample and standard, pipette 200 uL of the working solution into a clear 0.5 mL PCR tube.
4. Into one tube, pipet 10 uL of RNA Dilution Buffer (0 ng/uL).
5. Into a second tube, pipet 10 uL of RNA Broad Range Standard (100 ng/uL).
6. Pipette 10 uL of each RNA 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 RNA. Choose the Broad Range assay.
9. Follow the prompts on the screen, and first read the tube containing RNA Dilution Buffer (ie, Standard 1) and then read the tube containing RNA Broad Range Standard (ie, 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.

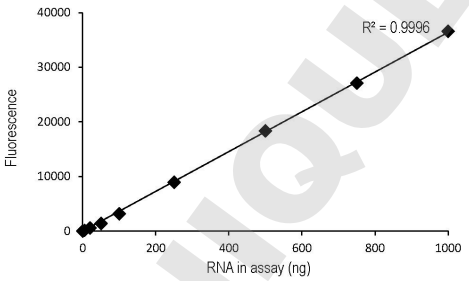

Figure 2. Linearity of AccuBlue® Broad Range RNA Quantitation assay between 5 and 1000 ng of RNA per well in microplate assay with excitation/emission at 630/670 nm.

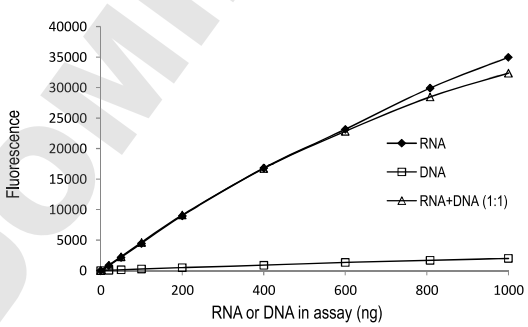

Figure 3. The AccuBlue® Broad Range RNA quantitation assay is highly specific for RNA over DNA. The presence of an equal amount of DNA in the sample has a negligible effect.

Table 1. Effect of common contaminants on AccuBlue® BR RNA assay signal

Compound	Initial concentration in RNA sample	Final concentration in assay (200 uL)	Result
Sodium Chloride	200 mM	10 mM	OK
Magnesium Chloride	40 mM	2 mM	NR
Sodium Acetate	40 mM	2 mM	OK
Ammonium Acetate	200 mM	10 mM	OK
Ethanol	2%	0.1%	OK
SDS	0.2%	0.01%	NR
Triton X-100	0.02%	0.001%	NR
CTAB	0.01%	0.0005%	NR
BSA	400 ug/mL	20 ug/mL	OK

OK means that there was less than a 10% change in fluorescence when the indicated amount of this contaminant was added to a sample. NR (not recommended) means that this contaminant caused more than 10% change in fluorescence when added to a sample at the given concentration.

Related Products

Catalog number	Product
CD201	RNAstorm™ Kit for Isolation of RNA from FFPE Tissue Samples
CD202	DNAstorm™ Kit for Isolation of RNA from FFPE Tissue Samples
31065	RNase-free Calf Thymus DNA, 1 mg/mL
41024	Water, Ultrapure Molecular Biology Grade, RNase-Free
22020	10X Phosphate-Buffer Saline (PBS), RNase-Free
31028	AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit
31006	AccuBlue® High Sensitivity dsDNA Quantitation Kit
31007	AccuBlue® Broad Range dsDNA Quantitation Kit
31060	AccuBlue® NextGen dsDNA Quantitation Kit
31069	AccuGreen™ Broad Range dsDNA Quantitation Kit (for Qubit®)
31066	AccuGreen™ High Sensitivity dsDNA Quantitation Kit (for Qubit®)
31045, 31046	Forget-Me-Not™ EvaGreen® qPCR Master Mix
31041, 31042	Forget-Me-Not™ EvaGreen® qPCR Master Mix, 2-Color Tracking
31043	Forget-Me-Not™ Universal Probe Master Mix
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in water
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in water
41028	Agarose LE, Ultra-Pure Molecular Biology Grade
41029	GelRed® Agarose LE
41030	GelGreen® Agarose LE
41006	TBE Buffer, 5X

Please visit our website at www.biotium.com for information on our life science research products including DNA quantitation kits, DNA and RNA extraction kits, environmentally friendly EvaGreen® qPCR master mixes, and other reagents for molecular and cell biology research.