SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

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

Product Information

AccuBlue® Broad Range RNA Quantitation Kit

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 uL
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

Store dye and buffers at 4°C. Store RNA Broad Range Standard at ≤ -20°C. Storage at -80°C will extend the shelf life of the RNA Broad Range 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 precautions when handling the dye, and dispose of the dye as hazardous chemical waste according to your local regulations.

Absorption/Emission: 648/669 nm (with RNA; Figure 1)

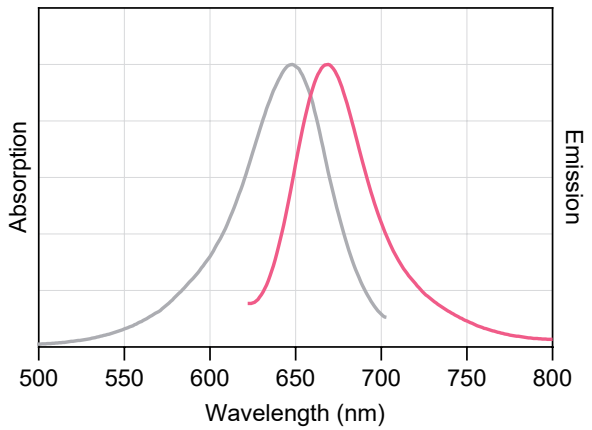

Figure 1. Excitation and emission spectra of RNA Broad Range Dye bound to RNA.

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 (Figure 2), which corresponds to sample concentrations of 0.5 to 100 ng/uL. Certain microplate readers are sensitive enough to extend the linear range down to 1 ng, or sample concentrations of 0.1 ng/uL (Figure 3; see Microplate Assay Protocol on pages 2-3 for details).

This kit is ideal to quantify 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 (dsDNA) and can tolerate an equimolar amount of dsDNA in the sample without significant effect on RNA quantitation (Figure 4). Purified RNA samples are still recommended. The assay may also be used to quantitate small RNAs such as miRNA (Figure 5), as well as single-stranded DNA (ssDNA), though ssDNA gives a lower fluorescence signal compared to RNA (Figure 6). The assay shows very low signal with dsRNA compared to total RNA (Figure 7).

The AccuBlue® Broad Range RNA Quantitation Kit 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® reader from Thermo Fisher, using the pre-programmed RNA Broad Range program (see Qubit® Assay Protocol on page 3).

Assay Considerations

- The RNA Broad Range Standard is made from total mammalian cell RNA. When quantifying RNA from non-mammalian sources, it may be preferable to use an RNA standard similar in size or source to your unknown samples.
- Precautions should be taken to avoid contamination with RNases. RNase contamination of any kit component 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.
- This assay can be performed with smaller reaction volumes than used in the protocol, but ratios of all reagents must be kept the same.

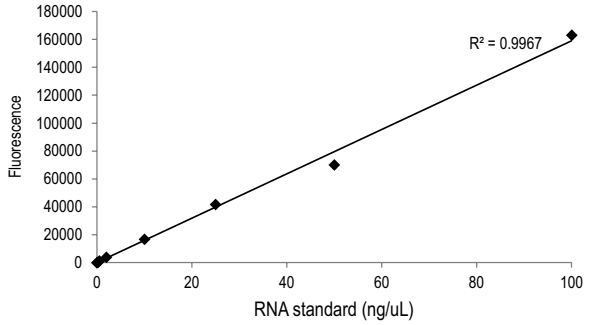

Figure 2. Linearity of AccuBlue® Broad Range RNA Quantitation assay between 0.1 ng/uL and 100 ng/uL of RNA per well in microplate assay with excitation/emission at 630/670 nm.

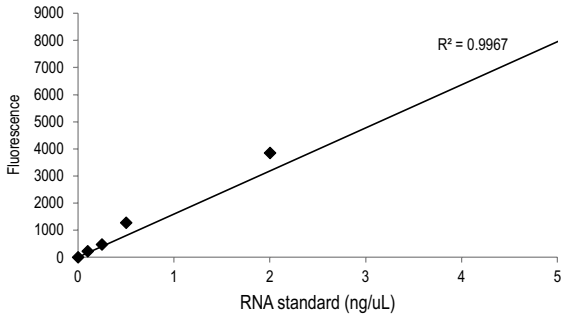

Figure 3. Detailed view of the low-end standards in the curve from Figure 2 showing the extension of the linear range to 0.1 ng/uL.

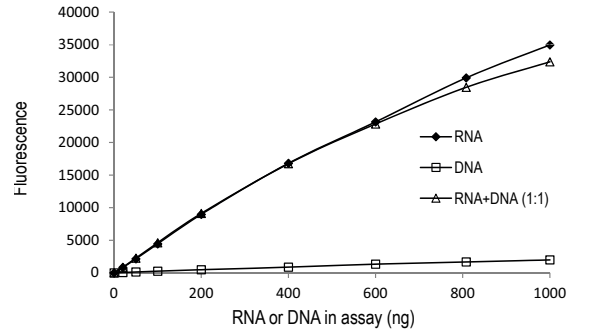

Figure 4. The AccuBlue® Broad Range RNA Quantitation assay is highly selective for RNA over dsDNA. The presence of an equal amount of dsDNA in the sample has a negligible effect.

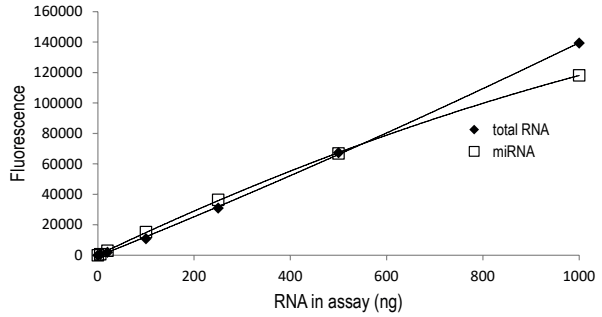

Figure 5. The AccuBlue® Broad Range RNA Quantitation assay can detect small RNAs like miRNA. Dilution series of a 20-mer small RNA was compared to the same concentrations of RNA Broad Range Standard (total Jurkat RNA). The fluorescence values were roughly similar, indicating that the assay is not highly dependent on RNA size. Note that at the highest concentrations miRNA fluorescence was lower than total RNA, but at the lower concentrations miRNA fluorescence was slightly higher.

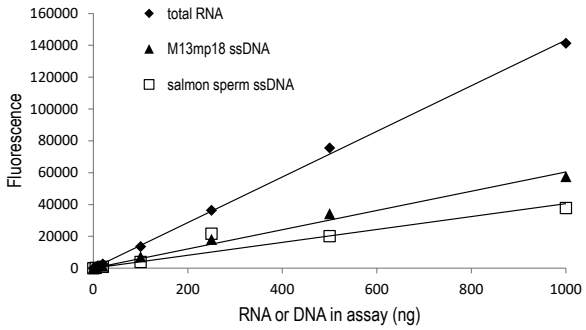

Figure 6. The AccuBlue® Broad Range RNA Quantitation assay detects ssDNA with lower signal than longer RNA. Dilution series of two types of ssDNA (M13mp18 linearized plasmid and salmon sperm DNA) were compared to the same concentrations of RNA Broad Range Standard (total Jurkat RNA).

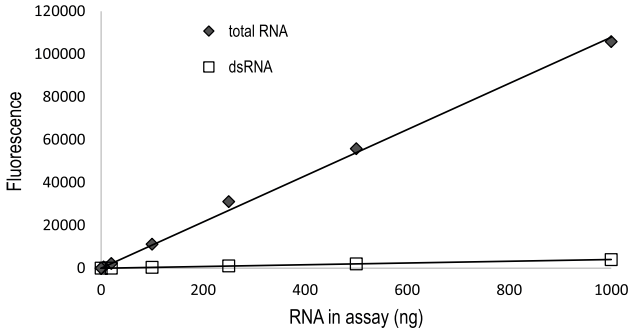

Figure 7. The AccuBlue® Broad Range RNA Quantitation assay gives a very low signal with double-stranded RNA (dsRNA) compared to total RNA. Dilution series of a dsRNA ladder (NEB) was compared to the RNA Broad Range Standard (total Jurkat RNA).

Microplate Assay Protocol

The linear range for this assay is dependent on the microplate reader. The typical linear range for this assay is 5-1000 ng, though certain microplate readers can extend the linear range down to 1 ng. This protocol provides directions on how to determine if your microplate reader is capable of detecting samples at 1 ng.

Materials required but not provided

- Black 96-well microplate

Microplate assay

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 of buffer to prepare enough working solution for an entire 96-well plate. Volumes may be scaled as required.

Microplate assay (continued)

3. Prepare RNA standards by diluting the 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.
   a. To perform the assay with a linear range of 5-1000 ng per 200 uL assay well, prepare standards for 0.5-100 ng/uL as shown in Table 1.
   b. Some microplate readers are sensitive enough to allow the linear range to be extended to 1 ng per 200 uL assay well. To test the extended range on your microplate reader, include the 0.25 ng/uL and 0.1 ng/uL RNA standards as shown in Table 1. Evaluate your data to determine whether the 0.25 ng/uL and 0.1 ng/uL standards are detectable above background and fall on the curve trendline. If either standard is undetectable or is not linear, then do not include these standards for assays on your instrument.
4. Pipette 200 uL of 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 at least 2 minutes in the dark.
6. Measure fluorescence using a microplate reader set to excitation/emission maxima of 630/670 nm 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 per 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 trend line to calculate the amount of unknown RNA in each well (y = fluorescence and x = ng RNA per well).

Table 1. Preparation of RNA standards

Standard	Volume of RNA	Volume of RNA Dilution Buffer
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	4 uL of 10 ng/uL	76 uL
*0.25 ng/uL	20 uL of 0.5 ng/uL	20 uL
*0.1 ng/uL	8 uL of 0.5 ng/uL	32 uL
0 ng/uL	0 uL	100 uL

*These standards are only required to extend the linear range (see Microplate Assay step 3b).

Qubit® Assay Protocol

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 other Qubit® models. This protocol may work on other handheld fluorometers, though customers should verify the excitation and emission on the instrument before proceeding.

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. Samples even slightly below 0.5 ng/uL will return the error message “Out of Range”. Therefore, use samples above 0.5 ng/uL for best results.

Materials required but not provided

- 0.5 mL clear tubes

Qubit® Assay

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 of buffer to prepare enough working solution for 10 tubes. Volumes can be scaled as required.
3. For each RNA standard or RNA sample, pipette 200 uL of the working solution into a clear 0.5 mL tube.
4. Into one tube, pipette 10 uL of RNA Dilution Buffer (0 ng/uL).
5. Into a second tube, pipette 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 for at least 2 minutes in the dark.
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. First, read the tube containing RNA Dilution Buffer (Standard 1), then, read the tube containing RNA Broad Range Standard (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.

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

Cat. No.	Product
28001	ExoBrite™ EV Total RNA Isolation Kit
CD504	CELLDATA RNAstorm™ Fresh Cell and Tissue RNA Isolation Kit
CD506	CELLDATA RNAstorm™ 2.0 FFPE RNA Extraction Kit
CD508	CELLDATA DNAstorm™/RNAstorm™ 2.0 Combination Kit
CD510-96	CELLDATA RNAstorm™ 2.0 MagBead FFPE RNA Extraction Kit
31086	RNaseReveal™ Activity Assay Kit
41044	EMBER™ Ultra RNA Gel Kit
41043	EMBER™ Ultra DNA Gel Kit
41032	EMBER500™ RNA Prestain Loading Dye
22028	RNase-X™ Decontamination Solution
31000, 31019	EvaGreen® Dye
31077	EvaGreen® Plus Dye, 20X in Water
31041, 31042	Forgot-Me-Not™ EvaGreen® qPCR Master Mix (2-Color Tracking)
31045, 31046	Forget-Me-Not™ EvaGreen® qPCR Master Mix (Low ROX or High ROX)
31028	AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit with DNA Standards
31060	AccuBlue® NextGen dsDNA Quantitation Kit
31006	AccuBlue® High Sensitivity dsDNA Quantitation Kit with DNA Standards
31007	AccuBlue® Broad Range dsDNA Quantitation Kit with DNA Standards
31066	AccuGreen™ High Sensitivity dsDNA Quantitation Kit
31069	AccuGreen™ Broad Range dsDNA Quantitation Kit
41001-41003	GelRed® Nucleic Acid Gel Stain
41004-41005	GelGreen® Nucleic Acid Gel Stain
41020	DNAzure® Blue Nucleic Acid Gel Stain, 100X
41029	GelRed® Agarose LE
41030	GelGreen® Agarose LE
41041, 41042	Precast GelRed® Agarose Gels, 1% Agarose/TAE

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