Revised: October 27, 2021

Product Information

EvaRuby™ Dye, 20X in Water

Catalog Number: 31079-T, 31079

Unit Size:
31079-T: 500 uL
31079: 2 x 1 mL

Spectral Properties
Ex/Em = 480/613 nm (with DNA; see Figure 1)

Storage and Handling
Store at -20°C, protected from light. EvaRuby™ Dye is very stable and may also be stored at 4°C. However, because this product is preservative-free, we recommend storing at -20°C to avoid contamination. Product is stable for at least 12 months from the date of receipt when stored as recommended. No information is available concerning the potential hazards of EvaRuby™ Dye. Exercise universal laboratory safety precautions during handling, and dispose as hazardous chemical waste.

Product Description
EvaRuby™ Dye is the first visible red intercalating dye for qPCR and HRM®. The dye is essentially non-fluorescent by itself, but becomes fluorescent upon binding to dsDNA, allowing it to be used as a typical intercalating dye for qPCR, HRM®, and LAMP. The dye's unique spectral properties allow it to be combined with commonly used fluorescent qPCR probes with little cross-talk in other fluorescence channels. Thus, EvaRuby™ Dye can be incorporated into probe-based qPCR assays with minimal interference with probe emission or assay efficiency. The dye can be added along with the other reaction components during set up, expanding the capabilities of probe-based qPCR to include real-time troubleshooting and post-PCR high-resolution melt analysis.

EvaRuby™ requires detection using a custom qPCR detection channel. Your qPCR instrument must have ~470 nm excitation and ~610 nm emission filters, as well as the option to acquire data using this non-standard filter combination. Instruments with paired excitation/emission filters may not be compatible with the dye. See Table 1 for more information on compatible instruments.

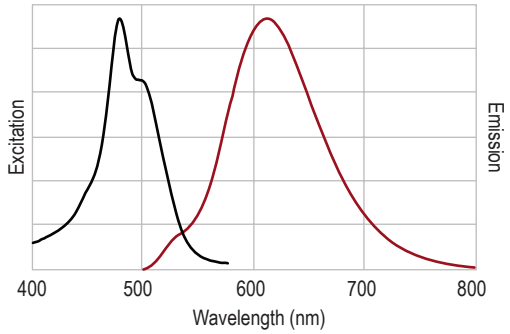

Figure 1. Excitation (left) and emission (right) spectra of EvaRuby™ Dye bound to dsDNA in TE buffer, pH 7.4.

Table 1. Instrument Compatibility Guidelines

Predicted compatibility is based on instrument documentation. We recommend contacting the manufacturer of your instrument to confirm compatibility before attempting to use EvaRuby™ Dye.

Compatibility	PCR Instrument
Validated:	Applied Biosystems: QuantStudio™ 5 96-well *Calibration required. Detect EvaRuby™ in x1_m4. For normalization, use VeriFluor™ Far-Red Passive Reference Dye (Cat. no. 29087) with x5_m5 channel.* Qiagen: Rotor-Gene® Q 5-Plex HRM *No calibration required. Detect EvaRuby™ in 470/610 nm channel. Normalization with VeriFluor™ Far-Red Passive Reference Dye (Cat. no. 29087) in the red 625/660 nm channel is optional.*
Expected: *Filter set available with option to select non-standard combination.*	Analytik Jena: qTOWER³, qTOWER³ G, qTOWER³ Touch & G Touch, qTOWER³ 84 & 84 G Applied Biosystems: 7500, 7500 Fast, QuantStudio™ 7 Pro, 7 Flex, 12K, & ViiA™7 BioRad: iCycler™ iQ, MyiQ™ 2 & 5, Chromo 2 & 4 Qiagen: Rotor-Gene® Q 6-Plex, Rotor-Gene® 3000 & 6000, Rotor-Gene® MDx Stratagene (Agilent): MX3005P Roche: LightCycler® 480
Partial: *Filter selection limits use to dye-only qPCR and melt analyses.*	BioRad: MiniOpticon™ Eppendorf: Mastercycler® Realplex 4 Roche: LightCycler® 2.0 & Nano
Incompatible: *Filter set unavailable or does not permit non-standard filter combination*	Applied Biosystems: 5700, 7300, 7000, 7700, 7900, StepOne™, StepOnePlus™; QuantStudio™ 1, 3, 5 384-well, 6 Pro, 6 Flex, & 3D BioRad: iCycler™, MyiQ™, CFX-96 Touch™, CFX-384 Touch™, CFX Connect™, QX100 & 200 Cepheid: SmartCyler®, SmartCyler® II Eppendorf: Mastercycler® Realplex 2 Fluidigm: Biomark Illumina: Eco™ RealTime PCR System Qiagen: Rotor-Gene® Q 2-Plex, QIAcuity, QIAquant Roche: LightCycler® 96 Stratagene (Agilent): AriaMx, MX3000P Stilla Technologies: Naica

General Considerations

- Before use, confirm that the ~470 nm excitation filter and ~610 nm emission filter are present and that the instrument is capable of acquiring qPCR reporter signal using this non-standard filter combination (See Table 1).
- If required, calibrate qPCR instrument following manufacturer protocols. The dye concentration required for calibration will vary by instrument. EvaRuby™ is an intercalating qPCR dye. Therefore, when calibrating, dye dilutions should be created using a solution of dsDNA in TE buffer to generate fluorescence. EvaRuby™ Dye at 0.9X with 60 ng/uL dsDNA in TE worked well for the Applied Biosystems® QuantStudio™ 5 96-well qPCR instrument.
- A 1X final dye concentration is suitable for both dye-based and probe-based qPCR but may not be optimal for all applications or qPCR instruments.
- EvaRuby™ Dye can be used for high resolution melting (HRM®) analysis, but not all qPCR instruments and software support HRM® in a custom channel. If possible, duplicate the HRM® temperature acquisition rates into a standard melt in the EvaRuby™ custom channel. Analysis can be performed with HRM® software, such as uAnalyze℠ (https://dna-utah.org). Follow your qPCR system's instructions for data collection and analysis.
- If your qPCR instrument requires a passive reference dye, use VeriFluor™ Far-Red (Cat. no. 29087) for optimal results. VeriFluor™ Far-Red is detected in the red (Cy®5 or Mustang Purple™) fluorescence channel. Do not use Cy®5 or a similar probe fluorophore if using VeriFluor™ Far-Red. High ROX concentrations (~500 nM) are incompatible with EvaRuby™. Using low ROX concentrations (~50 nM) for normalization is suboptimal and will increase background in the EvaRuby™ channel.
- EvaRuby™ can reduce the background fluorescence emission generated by unbound probes in early qPCR cycles (Figure 3). Quenching of probe background fluorescence may result in a lower threshold and earlier Ct values. The effect is dye dependent, with FAM most affected, while other probe dyes (Quasar® 670, CAL Fluor® Orange 560, & CAL Fluor® Red 610) exhibit virtually no change.

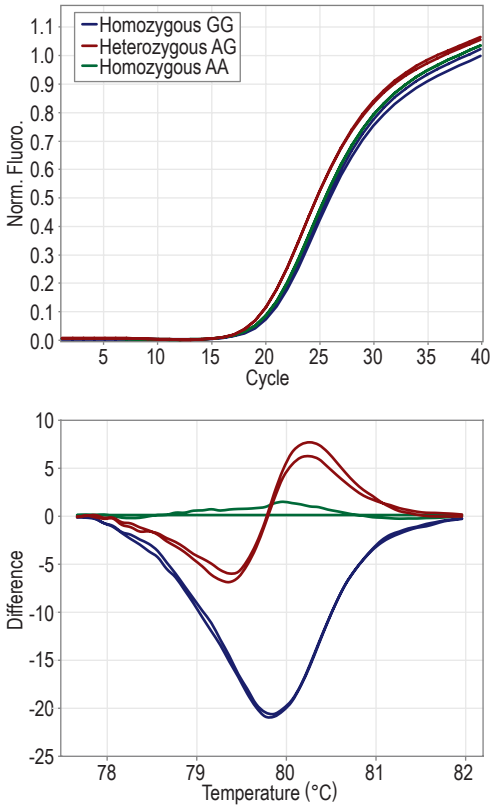

Figure 2. Normalized qPCR assay with the associated High Resolution Melt data difference plot. EvaRuby™ Dye at 1X in Forget-Me-Not™ Universal Probe qPCR Master Mix (Cat. No. 31043) was used to detect the Applied Biosystems® MeltDoctor™ HRM Positive Control Kit. Data was acquired using a Qiagen® Rotor-Gene® Q, and data analysis was performed with uAnalyze℠ baseline subtraction. EvaRuby™ Dye can clearly distinguish each homozygous variant as well as the heterozygous variant.

Protocol for qPCR

The following is an example protocol and reaction setup for qPCR using Biotium's 2X Forget-Me-Not™ Universal Probe qPCR Master Mix (Cat. no. 31043). Reaction conditions may require optimization for different applications.

Table 2. Example Reaction Setup

Reaction component	Amount required per 20 uL reaction	Final concentration
2X Forget-Me-Not™ Universal Probe qPCR Master Mix	10 uL	1X
20X EvaRuby™ Dye	1 uL	1X
Forward and Reverse Primers	Variable	0.1 - 0.9 uM each [a]
Fluorogenic Probe	Optional	0.1 - 0.5 uM [a]
Template DNA	Variable	See note [b]
400X VeriFluor™ Far-Red Passive Reference Dye	Optional	1X
PCR Grade Water	Add to 20 uL	

[a] Primers and probes should be designed using programs such as Primer3 (http://primer3.sourceforge.net/) or Primer Express® (Applied Biosystems). The optimal primer and probe concentrations should be determined empirically; primer concentrations of 200-400 nM and probe concentrations of 100-200 nM are generally suitable for most applications.

[b] The optimal amount of template DNA varies by application. Recommended amounts of genomic DNA template per reaction typically ranges from 50 pg to 50 ng per reaction. For two-step RT-qPCR, do not attempt to quantify cDNA after RT reaction by A_{260} measurement. Add diluted or undiluted RT reaction mix (from <1 ug RNA starting material) to the qPCR reaction, using a volume that is 10% or less of the final qPCR reaction volume.

1. Warm up the 20X EvaRuby™ solution to room temperature and thoroughly mix the solution by vortexing, dye may adhere to the vial during storage.
2. Perform real-time PCR on a qPCR instrument and acquire EvaRuby™ fluorescence signal at the annealing or extension step using a ~470 nm excitation filter and ~610 nm emission filter. If using probe or passive reference dye, acquire in additional channels as needed.
3. After PCR with EvaRuby™ Dye, PCR products do not need to be stained with another DNA gel stain for gel electrophoresis. Simply add DNA loading buffer to your PCR reaction solution, load on a gel, and conduct electrophoresis as usual. Gel visualization can be carried out using a 254 nm UV box with an EtBr filter or a blue LED imager. Alternatively, the gel may be imaged using a 488 nm laser-based gel scanner.

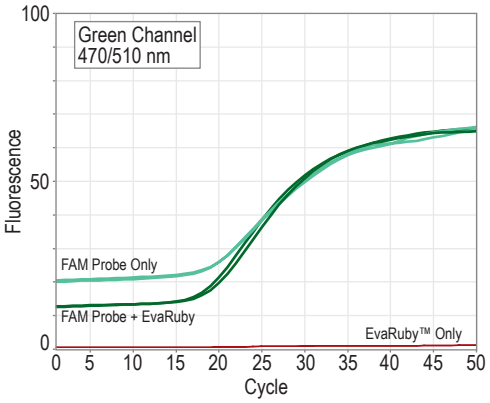

Figure 3. Comparison of reporter signals in the green channel of the Qiagen® Rotor-Gene® Q qPCR instrument. In this channel, the reactions containing FAM hydrolysis probes generate a robust signal, while those containing EvaRuby™ without FAM probe have negligible cross-talk in the green channel. In reactions containing both EvaRuby™ and probe, the signal generated by unbound FAM probe in early cycles is reduced compared to reactions with probe only. However, the maximum fluorescence signal in later cycles is unchanged, indicating that quenching is limited to early cycles. This phenomenon appears to be favorable to probe dyes, particularly FAM, and may result in lower thresholds and earlier target detection.

Related Products

Catalog number	Product
31043	Forget-Me-Not™ Universal Probe qPCR Master Mix
29087	VeriFluor™ Far-Red Passive Reference Dye, 400X in Water
41024-4L	Water, Ultrapure Molecular Biology Grade (4L Cubitainer®)
29052	ROX Reference Dye, 25 uM in TE Buffer
41006	TBE Buffer, 5X (4L Cubitainer®)
41028	Agarose LE, Ultra-Pure Molecular Biology Grade
41001	GelRed® Nucleic Acid Gel Stain, 3X in Water
41029	GelRed® Agarose LE
41030	GelGreen® Agarose LE
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in Water
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in Water
41011	GelRed® Prestain Plus 6X DNA Loading Dye
31022	Ready-to-Use 1 kb DNA Ladder
31032	Ready-to-Use 100 bp DNA Ladder
31030	DNA Gel Extraction Kit
29050	Cheetah™ HotStart Taq DNA Polymerase
31077	EvaGreen® Plus Dye, 2000X in DMSO
31000	EvaGreen® Dye, 20X in Water
31041	Forget-Me-Not™ EvaGreen® qPCR Master Mix (2-Color Tracking)
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®)
31073	AccuBlue® Broad Range RNA Quantitation Kit
CD201	RNAstorm™ Kit for Isolation of RNA from FFPE Tissue Samples
CD202	DNAstorm™ Kit for Isolation of RNA from FFPE Tissue Samples

Please visit our website at www.biotium.com for information on our life science research products, including VeriFluor™ Far-Red Passive Reference Dye, AccuBlue® and AccuClear® DNA quantitation, One-Step protein gel stains, fluorescent CF® Dye antibody conjugates, apoptosis reagents, fluorescent probes, environmentally friendly qPCR master mixes, and other reagents for molecular and cell biology research.