

# Product Information

## VeriFluor™ Far-Red Passive Reference Dye, 400X in Water

### Product List

Catalog no.	Dye	Unit size
29087-50 uL	VeriFluor™ Far-Red Passive Reference Dye	1 X 50 uL
29087-500 uL	VeriFluor™ Far-Red Passive Reference Dye	1 X 500 uL

### Storage and Handling

Store at -20°C, protected from light. Product is stable for at least 12 months from date of receipt if stored as recommended.

20X working solutions may be prepared in water or buffer. Solutions can be aliquoted and stored protected from light at -20°C, for at least 12 months.

**Color and Form:** Blue solution

**Absorption/Emission:** ~640/660 nm

### Product Description

VeriFluor™ Far-Red Passive Reference Dye is a far-red dye that can be used to normalize the fluorescent reporter signal during qPCR. It is an alternative to normalization with ROX passive reference dye, and a direct replacement for Mustang Purple™.

Unlike qPCR reporter dyes, which increase in fluorescence intensity as PCR product accumulates, a passive reference dye maintains a steady signal throughout the qPCR reaction. This steady signal is then used by qPCR instruments to compensate for well-to-well optical variations and normalization of the reporter dye signal. Typically, ROX passive reference dye is used. However, using ROX prevents the use of the orange emission channel for target detection in multiplex assays. VeriFluor™ Far-Red uses the red or Mustang Purple™ channel for normalization, freeing up the ROX channel for detection of probes labeled with dyes like JUN™, Texas Red®, or Cy®3.5. VeriFluor™ also can be used with Biotium's novel EvaRuby™ DNA intercalating dye for qPCR and HRM.

VeriFluor™ Far-Red is spectrally similar to Mustang Purple™ and can usually be used as a drop-in replacement by selecting the Mustang Purple™ calibration profile. It pairs exceptionally well with our Forget-Me-Not™ Master Mixes but also performs equally well with other commercially available master mixes. Additionally, VeriFluor™ Far-Red is insensitive to DTT, a reducing agent commonly present in forensic DNA extractions. Conversely, Mustang Purple™ has been reported to be quenched by DTT, resulting in normalization failure and overestimation of DNA (1).

### References

1) Forensic Sci Int Genet. 5. e5(2015).

### General Considerations

- VeriFluor™ Far-Red is detected in the red (Cy®5 or Mustang Purple™) fluorescence channel. If Cy®5 or a similar dye is used as a fluorophore, we recommend using ROX passive reference dye (Cat. no. 29052).
- A 1X final concentration is suitable for most qPCR instruments and master mixes. However, if using template tracking dye, a higher final concentration may be needed to compensate for the presence of the tracking dye.
- While we have found that VeriFluor™ Far-Red Passive Reference Dye may be used with the existing Mustang Purple™ calibration profile, we recommend performing a custom dye calibration for the highest possible precision. If the instrument has not been calibrated for Mustang Purple™, you may need to perform calibration using VeriFluor™ Far-Red. Refer to your qPCR instrument manual for calibration instructions.

### Protocol for Use as Passive Reference in qPCR

A 1X final concentration is suitable for most qPCR instruments. Below is a suggested dilution series for easy reaction set up.

1. Dilute 400X VeriFluor™ Far-Red Passive Reference Dye 1:20 by adding 1 uL VeriFluor™ Far-Red into 19 uL PCR grade water to obtain a 20X working solution.
2. Add 1 uL 20X working solution for each 20 uL of qPCR reaction mix. Or add 2.5 uL undiluted 400X VeriFluor™ Far-Red Passive Reference Dye per 1 mL tube of master mix.

**Note:** If using 2X Forget-Me-Not™ EvaGreen® qPCR Master Mix (Cat. no. 99801) with 40X Template Buffer (Cat. no. 99802), add 3 uL 20X VeriFluor™ Far-Red working solution for each 20 uL of qPCR reaction mix. Or add 7.5 uL undiluted 400X VeriFluor™ Far-Red per 1 mL tube of master mix.

3. Perform qPCR as usual. Select the VeriFluor™ Far-Red custom calibration profile or Mustang Purple™ as the passive reference. Alternatively, use the red channel for normalization.

### Related Products

Catalog number	Product
41024-4L	Water, Ultrapure Molecular Biology Grade (4L Cubitainer®)
31045, 31046	Forget-Me-Not™ EvaGreen® qPCR Master Mix (Low ROX or High ROX)
31041, 31042	Forget-Me-Not™ EvaGreen® qPCR Master Mix, (2-Color Tracking)
31043, 31044	Forget-Me-Not™ Universal Probe qPCR Master Mix
31078	N-Flux™ 5X Digital PCR Master Mix
31019	EvaGreen® Dye, 2000X in DMSO
31077	EvaGreen® Plus Dye, 20X in Water
31079	EvaRuby® Dye, 20X in Water
29050	Cheetah™ HotStart Taq DNA Polymerase, 500 U
29052	ROX reference dye, 25 uM in TE Buffer
41001	GelRed® Nucleic Acid Gel Stain, 3X in Water
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in Water
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in Water
41029	GelRed® Agarose LE
41030	GelGreen® Agarose LE
41006	TBE Buffer, 5X (4L Cubitainer®)

Please visit our website at [www.biotium.com](http://www.biotium.com) for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, ready-to-use ladders, fluorescent CF® dye antibody conjugates and reactive dyes, fluorescent probes, and kits for cell biology research.

Practicing real-time PCR may require additional licensing from Roche or Applied Biosystems.

EvaGreen Dye and Cheetah HotStart Taq DNA Polymerase are covered under US and international patents. Mustang Purple, JUN, and Texas Red are trademarks or registered trademarks of Thermo Fisher Scientific. Cy Dye is a registered trademark of GE Healthcare. Cubitainer is a registered trademark of Hedwin Corporation.

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