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ProductInformation

Reference Dye for Quantitative PCR

Product Code: **R 4526**
Storage Temperature 2-8 °C

Product Description

Sigma's Reference Dye for quantitative PCR is a proprietary dye for use with real-time PCR. It is used for normalization of reaction data when using SYBR Green, molecular probes, or dual-labeled probe chemistries for real-time detection. Maximum excitation of this dye is 586 nm and maximum emission is 605 nm. Instrument settings for ROX reference dye are satisfactory for the measurement of Reference Dye for Quantitative PCR.

Reagents

- Reference Dye for Quantitative PCR, Product Code R 4526, 100X dye. Provided as a 0.3 ml vial.

Materials and Reagents Required but not Provided

- SYBR Green JumpStart Taq ReadyMix, Product Code S4438 for SYBR Green detection
- JumpStart Taq ReadyMix for Quantitative PCR, Product Code D 7440 for use with molecular beacons or dual-labeled probe detection
- Water, PCR reagent, Product Code W 1754
- Primers
- DNA template
- Thermal cycler for quantitative PCR

Storage/Stability

Store Reference Dye for Quantitative PCR at 2-8 °C.

Precautions and Disclaimer

Reference Dye for Quantitative PCR is for R&D use only. Not for drug, household or other uses.

Procedure

The Reference Dye is supplied at 100x concentration, i.e., 0.5 µl should be used for the recommended 50 µl reaction. The dye may also be added to a master mix for subsequent aliquots of smaller reaction volumes.

For use with SYBR Green JumpStart Taq ReadyMix:

1. Add the following reagents to the proper thermal cycler reaction tube or plate:

Amount	Component
25 µl	SYBR Green JumpStart Taq ReadyMix
0.5 µl	Reference Dye for Quantitative PCR
- µl	Forward primer, 0.2 µM final concentration
- µl	Reverse primer, 0.2 µM final concentration
- µl	Template DNA
- µl	Water
50 µl	Total volume

Note: A template-primer master mix is recommended when performing multiple PCR reactions.

2. Mix gently by vortexing and briefly centrifuge to collect all components at the bottom of the tube.

3. Optimum cycling parameters vary with PCR composition and thermal cycler. It may be necessary to optimize the cycling parameters to achieve maximum product yield and/or quality.

Typical cycling parameters for 100- 600 bp fragments:

Initial denaturation	94 °C	2 min
40 cycles:		
Denaturation	94 °C	15 sec
Annealing	60 °C	1 min
Extension	72 °C	1 min
Hold	4 °C	

References

1. Morrison, T. B., et al., Quantification of low-copy transcripts by continuous SYBR® Green I monitoring during Amplification. BioTechniques, **24,** 954-962 (1998).
2. Sambrook, J. et al. *Molecular Cloning: A. Laboratory Manual*, Third Edition, Cold Spring Harbor Laboratory Press, New York (2000). (Product Code M 8265)

† The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

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