BIO-RAD

SsoFast™ EvaGreen® Supermix with Low ROX

Catalog #	Supermix Volume	Kit Size
172-5210	2 ml (2 x 1 ml vials)	200 x 20 µl reactions
172-5211	5 ml (5 x 1 ml vials)	500 x 20 µl reactions
172-5212	10 ml (10 x 1 ml vials)	1,000 x 20 µl reactions
172-5213	20 ml (1 x 10 ml bottle)	2,000 x 20 µl reactions

For research purposes only.

Storage and Stability
Guaranteed for 12 months in a constant temperature freezer at –20°C protected from light. For convenience, this supermix can be stored at 4°C for up to six months. Repeated freezing and thawing of the supermix is not recommended.

Kit Contents
SsoFast EvaGreen supermix with low ROX is a 2x concentrated, ready-to-use reaction master mix containing all components, except primers and template, for real-time quantitative PCR (qPCR) on ROX-dependent real-time PCR instruments. It contains antibody-mediated hot-start Sso7d-fusion polymerase, dNTPs, $MgCl_2$, EvaGreen dye (fluorescent nucleic acid dye with spectral properties similar to SYBR® Green I and fluorescein), enhancers, stabilizers, and ROX (passive reference dye).

Instrument Compatibility
This supermix is compatible with Applied Biosystems' 7500 Fast (or Standard), StepOne, StepOnePlus (with Rox reference setting turned off), Life Technologies' ViiA 7 real-time PCR instruments, and Stratagene's Mx real-time PCR systems.

Reaction Mix Preparation and Thermal Cycling Protocol
1. Thaw SsoFast EvaGreen supermix with low ROX and other frozen reaction components to room temperature. Mix thoroughly, centrifuge briefly to collect solution at the bottom of tubes, and then store on ice protected from light.

2. Prepare (on ice or at room temperature) enough assay master mix for all reactions by adding all required components except the DNA template according to the following recommendations (Table 1).

Table 1. Reaction Setup*

Component	Volume per 20 µl Reaction	Volume per 10 µl Reaction	Final Concentration
SsoFast EvaGreen supermix with low ROX (2x)	10 µl	5 µl	1x
Forward and reverse primers	Variable	Variable	300–500 nM each
DNA template	Variable	Variable	cDNA: 10 ng–10 fg Genomic DNA: 50 ng–5 pg
H_2O	Variable	Variable	—
Total reaction mix volume	*20 µl*	*10 µl*	—

* Scale all components proportionally according to sample number and reaction volumes.

3. Mix the assay master mix thoroughly to ensure homogeneity and dispense equal aliquots into each qPCR tube or into the wells of a qPCR plate. Good pipetting practice must be employed to ensure assay precision and accuracy.

4. Add DNA samples (and DNase-free H_2O if needed) to the PCR tubes or wells containing assay master mix (Table 1), seal tubes or wells with flat caps or optically transparent film, and vortex 30 sec or more to ensure thorough mixing of the reaction components. Spin the tubes or plate to remove any air bubbles and collect the reaction mixture in the vessel bottom.

5. Program thermal cycling protocol on the real-time PCR instrument according to Table 2.

6. Load the PCR tubes or plate onto the real-time PCR instrument and start the PCR run.

7. Perform data analysis according to the instrument-specific instructions.

Table 2. Thermal Cycling Protocol

Real-Time PCR System	Setting/ Mode	Polymerase Activation + DNA Denaturation	Amplification			Melt Curve Analysis
			Denaturation at 95°C/98°C*	Annealing/ Extension + Plate Read at 55–60°C	Cycles	
ABI 7500, StepOne, StepOnePlus, 7900HT, and ViiA 7	Fast	30 sec at **95°C** for cDNA or 2–3 min at **98°C** for genomic DNA*	3-5 sec**	10–30 sec	35–40	65°C–95°C In 0.5°C increments 2–5 sec/step (or use instrument default setting)
	Standard			15–30 sec		
Stratagene Mx series	Fast			10–30 sec		

* 98°C is highly recommended for genomic DNA template to ensure complete denaturation.
** For GC-rich targets from genomic DNA, longer denaturation of up to 30 sec per cycle may be necessary.

Recommendations for Primer Design and Assay Optimization

- The qPCR cycling protocols have been optimized for assays with a primer Tm of 55–60°C designed using the open source Primer3 program (**http://frodo.wi.mit.edu/**) under its default settings. For assays designed using other tools, the primer Tm should be recalculated using Primer3
- To achieve the best performance, carrying out a thermal gradient to determine the optimal annealing/extension temperature is recommended

Quality Control

SsoFast EvaGreen supermix with low ROX demonstrates high PCR efficiency and linear resolution over a wide linear dynamic range. Stringent specifications are maintained to ensure lot-to-lot consistency. This product is free of detectable DNase and RNase activities.

Related Products

- Reverse transcription reagents for 2-step RT-qPCR: iScript™ reverse transcription supermix for RT-qPCR (170-8840), iScript advanced cDNA synthesis kit for RT-qPCR (170-8842), iScript cDNA synthesis kit (170-8890)

To learn more about Bio-Rad's complete solution for amplification, visit **www.bio-rad.com/amplification**.

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