SsoFast™ Probes Supermix

Catalog #	Supermix Volume	Kit Size
172-5230	2 ml (2 x 1 ml vials)	200 x 20 µl reactions
172-5231	5 ml (5 x 1 ml vials)	500 x 20 µl reactions
172-5232	10 ml (10 x 1 ml vials)	1,000 x 20 µl reactions
172-5233	20 ml (1 x 20 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 Probes supermix is a 2x concentrated, ready-to-use master mix containing all components, except primers, probes, and template, for probe-based real-time quantitative PCR (simplex and duplex). This supermix contains antibody-mediated hot-start Sso7d-fusion polymerase, dNTPs, $MgCl_2$, enhancers, and stabilizers.

Instrument Compatibility
This supermix is compatible with all Bio-Rad real-time PCR systems, Applied Biosystems' StepOne and StepOnePlus (with ROX reference setting turned off), and the Roche LightCycler 480, QIAGEN Rotor-Gene Q, Eppendorf Mastercycler EP realplex, and Stratagene Mx real-time PCR systems.

Reaction Mix Preparation and Thermal Cycling Protocol
1. Thaw SsoFast Probes Supermix 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.

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 Probes Supermix (2x)	10 µl	5 µl	1x
Forward and reverse primers	Variable	Variable	250–350 nM** each primer
Fluorogenic probe	Variable	Variable	150–250 nM each probe
DNA template	Variable	Variable	cDNA: 100 ng–100 fg Genomic DNA: 500 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.
** For duplex assays with large copy number differences, decreasing the primer concentration of the higher copy target to 150 nM and increasing lower copy target primers to 900 nM will help achieve optimal results.

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 the PCR 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/ Block	Polymerase Activation + DNA Denaturation at 95°C	Amplification		
			Denaturation at 95°C	Annealing/ Extension + Plate Read at 60°C*	Cycles
Bio-Rad® CFX96™, CFX384™, CFX96 Touch™, CFX384 Touch™, CFX Connect™ systems	Fast	30 sec for cDNA or 2–3 min for genomic DNA	5–15 sec	10–30 sec	35–40
Bio-Rad® iQ™5, MiniOpticon™, Chromo4™, MyiQ™	Standard			15–30 sec	
ABI StepOne, StepOnePlus (with ROX reference setting turned off)	Fast			10-30 sec	
	Standard			10–30 sec	
Roche LightCycler 480	Fast			10–30 sec	
	Standard			15–30 sec	
QIAGEN Rotor-Gene, Stratagene Mx series	Fast			10–30 sec	

* For challenging duplex assays longer annealing/extension time (up to 30 sec) should be used.

Recommendations for Primer and Probe Design

- For best qPCR efficiency, design assays targeting an amplicon size of 70–150 bp
- SsoFast Probes supermix and the qPCR cycling protocols have been optimized for assays with a primer Tm of 60°C designed using the open source Primer3 program (**http://frodo.wi.mit.edu/**) under its default settings, or using the Primer Express software by Applied Biosystems. For assays designed using other tools, the primer Tm should be recalculated using Primer3 for determining annealing/extension temperature. The probe's Tm must be 10°C higher than the calculated primer Tm

Quality Control

SsoFast Probes supermix 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 solutions for amplification, visit **www.bio-rad.com/amplification**.

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