SsoFast™ Probes Supermix With ROX

200 x 20 µl reactions (catalog #172-5250)
500 x 20 µl reactions (catalog #172-5251)
1,000 x 20 µl reactions (catalog #172-5252)
2,000 x 20 µl reactions (catalog #172-5253)

For research purposes only. Store at –20°C.

Storage and Stability
SsoFast probes supermix with ROX is stable for 12 months when stored in a constant temperature freezer at –20°C. For convenience, it may be stored at 2–8°C for up to 6 months. Repeated freezing and thawing of the supermix is not recommended.

Kit Contents
SsoFast probes supermix with ROX is a 2x concentrated, ready-to-use reaction cocktail containing all components, except primers, probes and template, for probe-based real-time quantitative PCR (qPCR) on the Applied Biosystems 7900HT Fast (or 7900HT) real-time PCR system. The mixture has been optimized to deliver maximum PCR efficiency, specificity, and sensitivity using fast or conventional cycling protocols for probe-based simplex or duplex qPCR. This reagent is compatible with Applied Biosystems TaqMan gene expression assays and TaqMan endogenous controls.

The unique Sso7d-fusion polymerase in the supermix enables fast cycling without affecting PCR sensitivity, efficiency, and reproducibility. The dsDNA-binding protein, Sso7d, stabilizes the polymerase-template complex, increases processivity, and provides greater speed and reduced reaction times than conventional PCR enzymes.

The antibody-mediated hot-start feature employed by the Sso7d-fusion polymerase sequesters enzymatic activity prior to the initial PCR denaturation step. Upon heat activation, the antibodies denature irreversibly, releasing the fully active DNA polymerase. This enables specific and efficient primer extension with the convenience of room temperature reaction assembly.

The ROX internal reference dye included in SsoFast probes supermix with ROX is used for normalization of fluorescent signal and to correct for well-to-well optical variations on the Applied Biosystems 7900HT real-time PCR system.

Reagent	Kit Size	Supermix Volume	Description
SsoFast probes supermix with ROX (lavender cap tube or clear bottle)	200 x 20 µl reactions	1.0 ml x 2	2x reaction buffer with dNTPs, hot-start Sso7d-fusion polymerase, $MgCl_2$, ROX internal reference dye, enhancers and stabilizers
	500 x 20 µl reactions	1.0 ml x 5	
	1,000 x 20 µl reactions	1.0 ml x 10	
	2,000 x 20 µl reactions	20 ml (bottle)	

Reaction Setup
Thaw all components at room temperature. Mix thoroughly by inverting the tube/bottle several times to ensure homogeneity as a concentration gradient may form during –20°C storage. Centrifuge to collect contents at the bottom of the tube.

Component	Volume Per Reaction	Final Concentration
SsoFast probes supermix with ROX	10 µl	1x
Forward primer*	Variable	150 to 900 nM*
Reverse primer*	Variable	150 to 900 nM*
Fluorogenic probe	Variable	150 to 250 nM
RNase/DNase-free water	Variable	--
DNA template	Variable	--
Total volume	**20 µl†**	**--**

* To achieve optimal results for duplex assays with large copy number differences, the primer concentration of the lower copy target should be used at a higher concentration (e.g., 900 nM), and the primer concentration of the higher copy target should be restricted to a lower concentration (e.g., 150 nM)
† For smaller reaction volumes (for example, 10–15 µl), scale all components proportionally.

Quality Control
SsoFast probes supermix with ROX is free of contaminating DNase and RNase. This product is tested to demonstrate >90% PCR efficiency and linear resolution over six orders of dynamic range in challenging duplex qPCR assays. Stringent specifications are maintained to ensure lot-to-lot consistency.

General Recommendations for Optimal Results

- Careful preparation of a reaction cocktail is crucial in qPCR applications to reduce pipetting errors and maximize assay precision
- Assemble all required components except the sample template and dispense equal aliquots into each reaction tube. Add the sample template to each reaction tube as the final step
- Mix thoroughly and centrifuge briefly to ensure that all components are at the bottom of the reaction tube
- Full activation of the hot-start Sso7d-fusion polymerase occurs within 30 seconds at 95°C. Longer initial denaturation times may be required for complete denaturation of genomic DNA (see tables)
- Use the Applied Biosystems Primer Express or the Primer3 program (http://frodo.wi.mit.edu/) under default setting for General Primer Picking Conditions to design real-time PCR assays with a Tm of 60°C (or higher for GC-rich targets) for primers and a Tm ~10°C higher for the probe. Use the calculated Tm as the annealing/extension temperature
- Amplicon size should be as short as possible, ideally between 60 and 150 base pairs
- Suggested input quantities of template for a 20 µl reaction: an amount of cDNA corresponding to 100 ng to 100 fg of total RNA or 500 ng to 5 pg of genomic DNA. For optimal quantification, a slightly narrower range of input nucleic acids should be used

Optimized Cycling Conditions for qPCR

Applied Biosystems 7900HT Fast Real-Time PCR System (96-well block; fast mode)

	cDNA or Plasmid DNA			Genomic DNA	
Cycling Step	**Temperature**	**Time**	**# Cycles**	**Temperature**	**Time**
Enzyme activation	95°C	20 sec	1	95°C	2 min
Denaturation	95°C	1 sec	30–40	95°C	1 sec
Annealing/Extension	60°C	20 sec*		60°C	20 sec*

* For challenging duplex assays, longer annealing/extension time (up to 60 sec) should be used.

Applied Biosystems 7900HT Real-Time PCR System (96-well block; standard mode)

	cDNA or Plasmid DNA			Genomic DNA	
Cycling Step	**Temperature**	**Time**	**# Cycles**	**Temperature**	**Time**
Enzyme activation	95°C	2 min	1	95°C	2 min
Denaturation	95°C	15 sec	30–40	95°C	15 sec
Annealing/Extension	60°C	60 sec		60°C	60 sec

Applied Biosystems 7900HT Real-Time PCR System (384-well block; standard mode)

	cDNA or Plasmid DNA			Genomic DNA	
Cycling Step	**Temperature**	**Time**	**# Cycles**	**Temperature**	**Time**
Enzyme activation	95°C	2 min	1	95°C	2 min
Denaturation	95°C	15 sec	30–40	95°C	15 sec
Annealing/Extension	60°C	60 sec		60°C	60 sec

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

Bio-Rad Laboratories
2000 Alfred Nobel Drive, Hercules, CA 94547
510-741-1000