BIO-RAD

SsoFast™ EvaGreen® Supermix

200 x 20 µl reactions (catalog #172-5200)
500 x 20 µl reactions (catalog #172-5201)
1,000 x 20 µl reactions (catalog #172-5202)
2,000 x 20 µl reactions (catalog #172-5203)
2,500 x 20 µl reactions (catalog #172-5204)
5,000 x 20 ul reactions (catalog #172-5205)

For research purposes only. Store at –20°C and protect from light.

Storage and Stability
SsoFast EvaGreen supermix is stable for 12 months when stored in a constant temperature freezer at –20°C, protected from light. 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 EvaGreen supermix is a 2x concentrated, ready-to-use reaction cocktail containing all components, except primers and template, for real-time quantitative PCR (qPCR). The mixture has been optimized to deliver maximum PCR efficiency, sensitivity, and robust fluorescent signal using fast or conventional cycling protocols for dye-based detection in qPCR. Highly specific amplification is crucial for successful qPCR, since EvaGreen dye binds to and detects any dsDNA generated during amplification. 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.

Sso7d-fusion Polymerase
The unique Sso7d-fusion polymerase in SsoFast EvaGreen 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 amplification protocols.

EvaGreen Dye
EvaGreen dye is a fluorescent nucleic acid dye with spectral properties similar to SYBR® Green I and fluorescein. Unlike SYBR® Green I, EvaGreen dye exhibits very low PCR inhibition, which makes it an excellent choice for fast qPCR protocols. Thus, it can be used at high concentrations to generate greater fluorescent signals and provide increased sensitivity, making it ideal for qPCR applications.

Reagent	Kit Size	Supermix Volume	Description
SsoFast EvaGreen supermix (red cap tube or clear bottle)	200 x 20 µl reactions	1.0 ml x 2	2x reaction buffer with dNTPs, Sso7d-fusion polymerase, $MgCl_2$, EvaGreen dye, 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)	
	2,500 x 20 µl reactions	5.0 ml x 5	
	5,000 x 20 µl reactions	5.0 ml x 10	

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 EvaGreen supermix	10 µl	1x
Forward primer	Variable	300–500 nM
Reverse primer	Variable	300–500 nM
RNase/DNase-free water	Variable	--
DNA template	Variable	--
Total volume	**20 µl**	**--**

Note: For smaller reaction volumes (for example, 10–15 µl), scale all components proportionally.

Quality Control
SsoFast EvaGreen supermix is free of contaminating DNase and RNase. This product is tested to demonstrate >90% PCR efficiency and linear resolution over seven orders of dynamic range. 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
- Replicate samples should be assembled as a master mix with a single addition of the sample template.
- 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 and higher temperatures (98°C) are required for complete denaturation of genomic DNA (see tables on the next page).
- Use the Primer3 program (http://frodo.wi.mit.edu/) under default settings to design PCR primers with a melting temperature of 60°C (or higher for GC-rich targets), and use the primer Tm as the annealing temperature.
- The recommended primer concentration for use with the SsoFast EvaGreen supermix is between 300 nM and 500 nM.
- Suggested input quantities of template are: an amount of cDNA corresponding to 50 ng to 50 fg of total RNA or 50 ng to 5 pg of genomic DNA.
- This product does not contain fluorescein. EvaGreen dye exhibits sufficient background fluorescence to perform dynamic well factor collection for data normalization on the iCycler iQ®, iQ™5, or MyiQ™ real-time PCR systems.

Optimized Cycling Conditions for qPCR
Bio-Rad CFX96™ and CFX384™ Real-Time PCR Systems

	cDNA or Plasmid DNA			Genomic DNA	
Cycling Step	**Temperature**	**Time**	**# Cycles**	**Temperature**	**Time**
Enzyme activation	95°C	30 sec	1	98°C	2 min
Denaturation	95°C	1–5 sec	30–40	98°C	1–5 sec
Annealing/extension	55–60°C	1–5 sec		55–60°C	1–5 sec
Melt curve	65–95°C (in 0.5°C inc.)	2–5 sec/step	1	65–95°C (in 0.5°C inc.)	2–5 sec/step

Bio-Rad iCycler iQ, MyiQ, and iQ5 Real-Time PCR Systems

	cDNA or Plasmid DNA			Genomic DNA	
Cycling Step	**Temperature**	**Time**	**# Cycles**	**Temperature**	**Time**
Enzyme activation	95°C	30 sec	1	98°C	2 min
Denaturation	95°C	5 sec	30–40	98°C	5 sec
Annealing/extension	55–60°C	10 sec		55–60°C	10 sec
Melt curve	65–95°C (in 0.5°C inc.)	10 sec/step	1	65–95°C (in 0.5°C inc.)	10 sec/step

Roche LightCycler LC480 Real-Time PCR System

	cDNA or Plasmid DNA			Genomic DNA	
Cycling Step	**Temperature**	**Time**	**# Cycles**	**Temperature**	**Time**
Enzyme activation	95°C	30 sec	1	98°C	2 min
Denaturation	95°C	5 sec	30–40	98°C	5 sec
Annealing/extension	55–60°C	20 sec		55–60°C	20 sec
Melt curve	65–95°C	Continuous	1	65–95°C	Continuous

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

NOTICE TO PURCHASER: LIMITED LICENSE

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