

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)

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.

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 ideal choice for fast qPCR protocols. Thus, it can be used at a high concentrations to generate greater fluorescent signals and provide increased sensitivity, making it ideal for several applications, including qPCR and high-resolution melting (HRM™) technology.

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.

Reagent	Kit Size	Supermix Volume	Description
SsoFast EvaGreen supermix (red cap tube or clear bottle)	200 x 20µl reactions	1.0 ml x 2	2x reaction buffer with dNTPs, Sso7d-fusion polymerase, MgCl ₂ , EvaGreen dye, and stabilizers
	500 x 20µl reactions	1.0 ml x 5	
	1,000 x 20µl reactions	1.0 ml x 10	
	2,000 x 20µl reactions	20 ml (bottle)	

Reaction Set Up

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	500 nM
Reverse primer	Variable	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 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 next page)

- Use the Primer3 program (<http://frodo.wi.mit.edu/>) under default settings to design PCR primers with melting temperatures of 60°C (or higher for GC-rich targets), and use the primer T_m as the annealing temperature.
- The recommended primer concentration for use with the SsoFast EvaGreen supermix is **500 nM**. The 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's CFX96 and CFX384 Real-Time PCR Systems

Cycling Step	cDNA or Plasmid DNA			Genomic DNA	
	Temperature	Time	# Cycles	Temperature	Time
Enzyme activation	95–98°C	30 sec	1	98°C	2 min
Denaturation	95–98°C	1–5 sec	30–40	98°C	1–5 sec
Annealing/Extension	60–65°C	1–5 sec		60–65°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's iCycler iQ, MyiQ, and iQ5 Real-Time PCR Systems

Cycling Step	cDNA or Plasmid DNA			Genomic DNA	
	Temperature	Time	# Cycles	Temperature	Time
Enzyme activation	95–98°C	30 sec	1	98°C	2 min
Denaturation	95–98°C	5 sec	30–40	98°C	5 sec
Annealing/Extension	60–65°C	10 sec		60–65°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

Applied Biosystems' 7500 Fast Real-Time PCR System

Cycling Step	cDNA or Plasmid DNA			Genomic DNA	
	Temperature	Time	# Cycles	Temperature	Time
Enzyme activation	95–98°C	30 sec	1	98°C	2 min
Denaturation	95–98°C	5 sec	30–40	98°C	5 sec
Annealing/Extension	60–65°C	15–30 sec		60–65°C	15–30 sec
Melt curve	65–95°C	continuous	1	65–95°C	continuous

Note: ROX passive reference dye (catalog #172-5858) must be added to a final concentration of 0.75x for use on this instrument.

Recommendations for HRM
Bio-Rad's CFX96 and CFX384 Real-Time PCR Systems

Cycling Step	Temperature	Time	# Cycles
Enzyme activation	98°C	2–3 min	1
Denaturation	98°C	1–5 sec	30–40
Annealing/Extension	60–65°C	1–5 sec	
HRM	75–95°C (in 0.2°C inc.)	5 sec/step	1

Note: SsoFast EvaGreen supermix cannot be used with bisulfite-converted DNA for methylation studies.

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

NOTICE TO PURCHASER: LIMITED LICENSE

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,079,352, 5,789,224, 5,618,711, 6,127,155, 5,677,152 (claims 1 to 23 only), 5,733,258 (claims 1 and 6 only), 5,407,800, 5,322,770, 5,310,652, 5,994,056, 6,171,785, and claims outside the US corresponding to US Patent No. 4,889,818. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim (such as apparatus or system claims in US Patent No. 6,814,934) and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Bio-Rad Laboratories, Inc. is licensed by Biotium Inc. to sell reagents containing EvaGreen dye for use in real-time PCR, for research purposes only.

SYBR Green is a registered trademark of Invitrogen, Inc. EvaGreen is a registered trademark of Biotium, Inc. HRM is a trademark of Corbett Life Science.