SsoAdvanced™ SYBR® Green Supermix

200 x 20 µl reactions (catalog #172-5260)
500 x 20 µl reactions (catalog #172-5261)
1,000 x 20 µl reactions (catalog #172-5262)
2,500 x 20 µl reactions (catalog #172-5264)
5,000 x 20 µl reactions (catalog #172-5265)

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 may be stored at 2–8°C for up to 6 months.
Repeated freezing and thawing of the supermix is not recommended.

SsoAdvanced™ SYBR® Green 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.

- **Universal cycling conditions** – robust formulation delivers consistent performance across a broad range of cycling conditions (standard and fast)
- **Short run times and time-to-results with uncompromised qPCR data quality** — optimized buffer and Sso7d fusion polymerase deliver fast reaction times via instant polymerase activation (antibody-mediated hot-start) and rapid polymerization kinetics
- **Minimal inhibition of PCR** — Sso7d fusion polymerase provides increased resistance to PCR inhibitors and ensures maximum efficiency, sensitivity, and reproducibility

Quality Control

SsoAdvanced™ SYBR® Green supermix demonstrates >90% PCR efficiency and linear resolution over seven orders of dynamic range. Stringent specifications are maintained to ensure lot-to-lot consistency. This product is free of detectable DNase and RNase activities.

Kit Contents

Reagent	Kit Size	Supermix Volume	Description
SsoAdvanced™ SYBR® Green supermix	200 x 20 µl reactions	1.0 ml x 2	2x reaction buffer with hot-start Sso7d-fusion polymerase, SYBR® Green dye, dNTPs, $MgCl_2$, enhancers, and stabilizers
	500 x 20 µl reactions	1.0 ml x 5	
	1,000 x 20 µl reactions	1.0 ml x 10	
	2,500 x 20 µl reactions	5.0 ml x 5	
	5,000 x 20 µl reactions	5.0 ml x 10	

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
SsoAdvanced™ SYBR® Green supermix	10 µl	1x
Forward primer	Variable	250–500 nM
Reverse primer	Variable	250–500 nM
RNase/DNase-free water	Variable	--
DNA template	Variable	cDNA: 100 ng - 100 fg gDNA: 50 ng - 5 pg
Total volume	**20 µl**	**--**

Note: For smaller or larger reaction volumes scale all components proportionally.

Protocol Recommendations for Fast and Standard Cycling

cDNA or Plasmid DNA			
Cycling Step	**Temperature**	**Time**	**# Cycles**
Enzyme activation/Initial DNA denaturation	95°C	30 sec	1
Denaturation	95°C	5 sec	35 to 40
Annealing/extension (for amplicons <250 bp and 40 to 60% GC)	Optimized annealing temperature	10–30 sec*	
Melt curve	65–95°C (in 0.5°C increments)	2–5 sec/step	1

*Shorter annealing/extension times (1-10 sec) can be used for amplicons <100 bp. Longer annealing/extension times (30–60 sec) can be used for amplicons >250 bp, GC- or AT- rich targets, or for higher input cDNA amounts (e.g.100 ng).

Genomic DNA (gDNA)			
Cycling Step	**Temperature**	**Time**	**# Cycles**
Enzyme activation/Initial DNA denaturation	98°C	2 min	1
Denaturation	98°C	5 sec	35 to 40
Annealing/extension (for amplicons <250 bp and 40 to 60% GC)	Optimized annealing temperature	10–30 sec**	
Melt curve	65–95°C (in 0.5°C increments)	2–5 sec/step	1

**Shorter annealing/extension times (1-10 sec) can be used for amplicons <100 bp. Longer annealing/extension times (30–60 sec) can be used for amplicons >250 bp, GC- or AT- rich targets, or for higher input gDNA amounts (e.g. 50 ng).

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 tube as the final step. Mix thoroughly and centrifuge briefly prior to cycling.
- Full activation of the hot-start Sso7d-fusion polymerase occurs within 30 sec at 95°C. Longer initial denaturation times and higher temperatures (98°C) are required for complete denaturation of genomic DNA.
- 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/extension temperature.
- For existing primers, determine the Tm using Primer3 under default settings and use it as the annealing/extension temperature.
- This product contains fluorescein.

Instrument Compatibility

SsoAdvanced™ SYBR® Green supermix is compatible with all Bio-Rad real-time PCR instruments, and the Roche LightCycler LC480, QIAGEN Rotor-Gene 6000, and Eppendorf Mastercycler EP realplex real-time PCR systems.

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

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