

SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix

Catalog #	Description
172-5016	SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix, 2 ml (2 x 1 ml vials), 200 x 20 µl reactions
172-5017	SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix, 5 ml (1 x 5 ml vial), 500 x 20 µl reactions
172-5018	SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix, 10 ml (2 x 5 ml vials), 1,000 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 up to 3 months.

Kit Contents

SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix is a 2x concentrated, ready-to-use reaction supermix optimized for challenging real-time PCR reactions on any real-time PCR instrument (ROX dependent and independent). It contains antibody-mediated hot-start Sso7d fusion polymerase, dNTPs, MgCl₂, SYBR® Green I, enhancers, stabilizers, and a blend of passive reference dyes (including ROX and fluorescein).

Instrument Compatibility

This supermix is compatible with all Bio-Rad and other commercially available real-time PCR systems.

Reaction Mix Preparation and Thermal Cycling Protocol

1. Thaw SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix and other frozen reaction components to room temperature. Mix thoroughly, centrifuge briefly to collect solutions at the bottom of tubes, then store on ice protected from light.
2. Prepare (on ice or at room temperature) enough assay master mix for all quantitative PCR (qPCR) reactions by adding all required components, except the DNA template, according to the recommendations in Table 1.

3. Mix the assay master mix thoroughly to ensure homogeneity and dispense equal aliquots into each PCR tube or into the wells of a PCR plate. Good pipetting practice must be employed to ensure assay precision and accuracy.
4. Add DNA samples (and nuclease-free H₂O, 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.

Note: DNA samples include purified DNA, cell lysates or tissue extracts, up to 20% undiluted cDNA, samples containing inhibitors including, but not limited to, heparin, hematin, polysaccharides, polyphenols, salts, humic acid, indigo dye, melanin, and cell culture media.

Note: Lysates and samples with known inhibitors may require an evaluation to determine the maximum input volume. Different crude samples present various levels of PCR inhibitors. This testing can be completed using a dilution series of the lysate to determine the point at which no inhibition occurs.

5. Program the thermal cycling protocol on a real-time PCR instrument according to Table 2.
6. Load the PCR tubes or plate into the real-time PCR instrument and start the PCR run.
7. Perform data analysis according to the instrument-specific instructions.

Table 1. Reaction setup.*

Component	Volume/20 µl Reaction, µl	Volume/10 µl Reaction, µl	Final Concentration
SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix (2x)	10	5	1x
Forward and reverse primers	Variable	Variable	250–500 nM each primer
DNA template (add at step 4)	Variable	Variable	cDNA: 100 ng–100 fg Genomic DNA: 50 ng–5 pg Lysate: Up to 5 µl in a 20 µl reaction and up to 2 µl in a 10 µl reaction
Nuclease-free H ₂ O	Variable	Variable	—
Total reaction mix volume	20	10	—

* Scale all components proportionally according to sample number and reaction volumes.

Table 2. Thermal cycling protocol.

Real-Time PCR System	Setting/Block	Polymerase Activation and DNA Denaturation	Amplification			Melt Curve Analysis
			Denaturation at 98°C, sec	Annealing/Extension and Plate Read at 60°C, sec	Cycles	
Bio-Rad® CFX96™, CFX384™, CFX96 Touch™, CFX96 Touch Deep Well, CFX384 Touch™, CFX Connect™	SYBR® only	2–3 min at 98°C	5–15	15–60	35–40	65–95°C 0.5°C increments at 2–5 sec/step (or use instrument default setting)
Bio-Rad® iQ™5, MiniOpticon™, Chromo4™, MyiQ™	Standard			15–30		
Applied Biosystems 7500 and 7900HT, QuantStudio, StepOne, StepOnePlus, ViiA 7	Fast			15–30		
	Standard			60		
Roche LightCycler 96 and 480	Fast			15–30		
	Standard			60		
QIAGEN Rotor-Gene and Stratagene Mx series	Fast	15–30				

Recommendations for Primer Design and Assay Optimization

- For best qPCR efficiency, design assays targeting an amplicon size of 70–150 bp
- The SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix and the qPCR cycling protocols have been optimized for assays with a primer melting temperature (T_m) of 60°C and designed using the open source Primer3, Primer3Plus, or Primer-BLAST programs with their default settings. If primers are designed using other programs, adjust the annealing temperatures accordingly.

Quality Control

SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green 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 activity.

Related Products

Reverse transcription reagents for two-step real-time qPCR:

- iScript™ Advanced cDNA Synthesis Kit for RT-qPCR (172-5038)
- iScript Reverse Transcription Supermix for RT-qPCR (170-8841)

Visit bio-rad.com/amplification for more information.

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