BIO-RAD

# SsoAdvanced™ Universal SYBR® Green Supermix

| Catalog # | Description |
|---|---|
| 1725270 | **SsoAdvanced™ Universal SYBR® Green Supermix**, 2 ml (2 x 1 ml vials), for 200 x 20 µl reactions |
| 1725271 | **SsoAdvanced™ Universal SYBR® Green Supermix**, 5 ml (5 x 1 ml vials), for 500 x 20 µl reactions |
| 1725272 | **SsoAdvanced™ Universal SYBR® Green Supermix**, 10 ml (10 x 1 ml vials), for 1,000 x 20 µl reactions |
| 1725274 | **SsoAdvanced™ Universal SYBR® Green Supermix**, 25 ml (5 x 5 ml vials), for 2,500 x 20 µl reactions |
| 1725275 | **SsoAdvanced™ Universal SYBR® Green Supermix**, 50 ml (10 x 5 ml vials), for 5,000 x 20 µl reactions |

Sso7d FUSION POLYMERASE EMPOWER YOUR PCR

**For research purposes only.**

## Introduction

SsoAdvanced™ Universal SYBR® Green Supermix is an exclusive high-performance real-time quantitative PCR (qPCR) reagent based on Bio-Rad's patented* Sso7d fusion protein polymerase technology and advanced buffer formulation. This supermix is uniquely formulated to provide higher processivity, increased PCR inhibitor tolerance, and robust performance with challenging templates and targets for qPCR using SYBR® Green.

## 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 for up to 3 months.

## Kit Contents

SsoAdvanced™ Universal SYBR® Green Supermix is a 2x concentrated, ready-to-use reaction supermix optimized for dye-based real-time PCR on any real-time PCR instrument (ROX independent and ROX dependent). It contains antibody-mediated hot-start Sso7d fusion polymerase, dNTPs, $MgCl_2$, SYBR® Green I Dye, enhancers, stabilizers, and a blend of passive reference dyes (including ROX and fluorescein).

## Instrument Compatibility

This supermix is validated with all Bio-Rad and ROX-dependent Applied Biosystems real-time PCR instruments, and with the Roche LightCycler 480, QIAGEN Rotor-Gene Q, Eppendorf Mastercycler ep *realplex*, and Stratagene Mx real-time PCR systems. This supermix should be compatible with other real-time PCR systems designed to detect SYBR® Green.

## Reaction Mix Preparation and Thermal Cycling Protocol

1. Thaw SsoAdvanced™ Universal 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 reaction mix for all qPCR reactions by adding all required components, except the DNA template, according to the recommendations in Table 1.

**Table 1. Reaction setup.***

| Component | Volume per 20 µl Reaction | Volume per 10 µl Reaction | Final Concentration |
|---|---|---|---|
| SsoAdvanced™ Universal SYBR® Green Supermix (2x) | 10 µl | 5 µl | 1x |
| Forward and reverse primers | Variable | Variable | 250–500 nM each |
| DNA template **(add at step 4)** | Variable | Variable | cDNA: 100 ng–100 fg Genomic DNA: 50 ng–5 pg |
| Nuclease-free $H_2O$ | Variable | Variable | — |
| **Total reaction mix volume** | **20 µl** | **10 µl** | **—** |

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

3. Mix the reaction mix thoroughly to ensure homogeneity and dispense equal aliquots into each qPCR tube or into the wells of a qPCR plate. Good pipetting practice must be employed to ensure assay precision and accuracy.
4. Add DNA samples (and nuclease-free $H_2O$, if needed) to the qPCR tubes or wells containing reaction 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.
5. Program the thermal cycling protocol on a real-time PCR instrument according to Table 2.
6. Load the qPCR tubes or plate into the real-time PCR instrument and start the PCR run.
7. Perform data analysis according to the instrument-specific instructions.

* U.S. patents 6,627,424; 7,541,170; and 7,560,260.

**Table 2. Thermal cycling protocol.**

| Real-Time PCR System | Setting/Mode | Polymerase Activation and DNA Denaturation | Amplification | | | Melt Curve Analysis |
|---|---|---|---|---|---|---|
| | | | Denaturation at 95°C/98°C | Annealing/ Extension and Plate Read at 60°C** | Cycles | |
| Bio-Rad® CFX96™, CFX384™, CFX96 Touch™, CFX96 Touch Deep Well, CFX384 Touch™, CFX Connect™ Systems | SYBR® only | 30 sec at **95°C or 98°C** for cDNA or 2–3 min at **98°C** for genomic DNA* | 5–15 sec | 15–30 sec | 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™, MyiQ2 Systems | Standard | | | 15–30 sec | | |
| ABI 7500, StepOne, StepOnePlus, 7900HT, and ViiA 7 | Fast | | | 15–30 sec | | |
| | Standard | | | 60 sec | | |
| Roche LightCycler 480 | Fast | | | 15–30 sec | | |
| | Standard | | | 60 sec | | |
| QIAGEN Rotor-Gene and Stratagene Mx series | Fast | | | 15–30 sec | | |

* 98°C is highly recommended for genomic DNA template to ensure complete denaturation.

** Shorter annealing/extension times (1–10 sec) can be used for amplicons <100 bp. Longer annealing/extension times (40–60 sec) can be used for amplicons >250 bp, GC- or AT-rich targets, crude samples, or for higher input amounts (for example 100 ng of cDNA or 50 ng of genomic DNA).

## Recommendations for Primer Design and Assay Optimization

- For best qPCR efficiency, design assays targeting an amplicon size of 70–150 bp
- The SsoAdvanced™ Universal SYBR® Green Supermix and the qPCR cycling protocols have been optimized for assays with a primer melting temperature ($T_m$) of 60°C 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 temperature accordingly

Visit **bio-rad.com/PrimePCR** for predesigned validated assays. To learn more about sample preparation, assay and experimental design, and troubleshooting, visit **bio-rad.com** and search for document #10031339.

## Quality Control

SsoAdvanced™ Universal 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 and RNase activities.

## Related Products

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

- iScript™ Reverse Transcription Supermix for RT-qPCR (1708840)
- iScript Advanced cDNA Synthesis Kit for RT-qPCR (1725037)
- iScript gDNA Clear cDNA Synthesis Kit (1725034)

Real-time qPCR supermix with SYBR® Green:

- iTaq™ Universal SYBR® Green Supermix (1725120)
- iTaq™ Universal SYBR® Green One-Step Kit (1725150)

Primer and probe assays for real-time qPCR:

- PrimePCR™ Assays and Panels

Visit **bio-rad.com/amplification** to learn more about Bio-Rad's complete solution for amplification.



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