BIO-RAD

# SsoAdvanced™ Universal Probes Supermix

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| Catalog # | Supermix Volume | Kit Size |
|---|---|---|
| 172-5280 | 2 ml (2 x 1 ml vials) | 200 x 20 µl reactions |
| 172-5281 | 5 ml (5 x 1 ml vials) | 500 x 20 µl reactions |
| 172-5282 | 10 ml (10 x 1 ml vials) | 1,000 x 20 µl reactions |
| 172-5284 | 25 ml (5 x 5 ml vials) | 2,500 x 20 µl reactions |
| 172-5285 | 50 ml (10 x 5 ml vials) | 5,000 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 for up to three months.

**Kit Contents**
SsoAdvanced universal probes supermix is a 2x concentrated, ready-to-use supermix optimized for probe-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, $MgCl_2$, enhancers, stabilizers, and a blend of passive reference dyes (including ROX and fluorescein).

**Instrument Compatibility**
This supermix is compatible with all Bio-Rad and ROX-dependent Applied Biosystems real-time PCR instruments, and with the Roche LightCycler LC480, Qiagen Rotor-Gene Q, Eppendorf Mastercycler ep realplex, and Stratagene Mx real-time PCR systems.

**Reaction Mix Preparation and Thermal Cycling Protocol**

1. Thaw SsoAdvanced universal probes supermix and other frozen reaction components to room temperature. Mix thoroughly, centrifuge briefly to collect solutions at the bottom of tubes, and then store on ice protected from light.

2. Prepare (on ice or at room temperature) enough reaction setup for all qPCR reactions by adding all required components *except the template* according to the following recommendations (Table 1).

**Table 1. Reaction setup***

| Component | Volume per 20 µl Reaction | Volume per 10 µl Reaction | Final Concentration |
|---|---|---|---|
| SsoAdvanced universal probes supermix (2x) | 10 µl | 5 µl | 1x |
| Forward and reverse primers | Variable | Variable | 250–900 nM** each |
| Fluorogenic probe | Variable | Variable | 150–250 nM each |
| Template **(add at step 4)** | Variable | Variable | cDNA: 100 ng–100 fg<br>Genomic DNA: 500 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.
** For duplex assays with large ΔCq ($\Delta C_T$) values, decreasing the primer concentrations for the higher expressing target may help. To validate, perform a primer matrix to determine optimal final primer concentration.

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 samples (and nuclease-free $H_2O$, if needed) to the PCR tubes or wells containing the reaction setup (Table 1), seal tubes or wells with flat caps or optically transparent film, and vortex 30 seconds 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 thermal cycling protocol on the real-time PCR instrument according to Table 2.

6. Load the PCR tubes or plate onto the real-time PCR instrument and start the PCR run.

7. Perform data analysis according to the instrument-specific instructions.

| Table 2. Thermal cycling protocol | | | | | |
|---|---|---|---|---|---|
| **Real-Time PCR System** | **Setting/ Scan Mode** | **Polymerase Activation and DNA Denaturation** | **Amplification** | | |
| | | | **Denaturation at 95°C** | **Annealing/ Extension + Plate Read at 60°C**** | **Cycles** |
| Bio-Rad® CFX96™, CFX384™, CFX96 Touch™, CFX384 Touch™, CFX Connect™ systems | All channels | 30 sec at **95°C** for cDNA<br>or<br>2–3 min at **95°C** for genomic DNA* | 5–15 sec | 10–30 sec | 35–40 |
| Bio-Rad® iQ™5, MiniOpticon™, Chromo4™, MyiQ™ | Standard | | | 15–30 sec | |
| AB 7500, StepOne, StepOnePlus, 7900HT and ViiA7 | Fast | | | 10–30 sec | |
| | Standard | | | 60 sec | |
| Roche LightCycler 480 | Fast | | | 10–30 sec | |
| | Standard | | | 60 sec | |
| Qiagen Rotor-Gene and Stratagene Mx series | Fast | | | 10–30 sec | |

* 2-3 min denaturation at 95°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 (30–60 sec or more) can be used for amplicons >250 bp, GC- or AT- rich targets, low expressing targets, crude samples, or for higher input amounts (for example,100 ng of cDNA or 500 ng of genomic DNA).

**Recommendations for Assay Design and Optimization**

- For best qPCR efficiency, design assays targeting an amplicon size of 70–150 bp
- The SsoAdvanced universal probes 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 under default settings. If primers are designed using other programs, adjust the annealing temperature accordingly
- The probe's $T_m$ should be 8–10°C higher than the calculated primer $T_m$. In a duplex reaction, applying the brighter fluorophores to the lower expressing targets and the less bright fluorophores to the higher expressing targets can help in visualizing data

To learn more about sample preparation, assay and experimental design including duplex optimization, and troubleshooting, visit **www.bio-rad.com** and search for item **10031340**.

**Quality Control**

SsoAdvanced universal probes 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 PCR:
  - iScript™ reverse transcription supermix for RT-qPCR (170-8840)
  - iScript advanced cDNA synthesis kit for RT-qPCR (170-8842)
- Real-time PCR supermix for probe-based qPCR:
  - iTaq™ universal probes supermix (172-5130)
- Real-time PCR one-step kit:
  - iScript one-step RT-qPCR kit for probes (170-8894)

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

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