SsoAdvanced™ PreAmp Supermix

Catalog #	Description
172-5160	**SsoAdvanced PreAmp Supermix**, 1.25 ml, 50 x 50 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 6 months.

Kit Contents

SsoAdvanced PreAmp Supermix is a 2x concentrated, ready-to-use reaction master mix optimized for unbiased target-specific preamplification of a limited amount of nucleic acid using up to 400 PrimePCR™ PreAmp SYBR® Green or Probe Quantitative PCR (qPCR) Assays as well as 100 TaqMan Assays (because of concentration limitations) or custom-designed assays. It contains antibody-mediated hot-start Sso7d fusion DNA polymerase, dNTPs, salts, enhancers, and stabilizers.

Prepare Preamplification Assay Pool

PrimePCR PreAmp Assays

Add 5 µl of each assay, up to 100 assays, and nuclease-free water to a total volume of 500 µl. Mix thoroughly, then centrifuge briefly to collect the solution at the bottom of the tube. Store on ice. This will provide enough assay pool for 100 preamplification reactions. Use 5 µl of each assay pool in a 50 µl preamplification reaction. Up to four pools may be added per amplification reaction.

20x TaqMan Assays

Add 5 µl of each assay, up to 100 assays, and nuclease-free water to a total volume of 500 µl. Mix thoroughly, then centrifuge briefly to collect the solution at the bottom of the tube. Store on ice. This will provide enough assay pool for 40 preamplification reactions. Use 12.5 µl of assay pool in a 50 µl preamplification reaction. Preamplification is limited to 100 assays because of concentration limitations.

Custom-Designed Assays

Because primer stocks may be at different starting concentrations, the assay pool, up to 100 assays, should be prepared such that the final preamplification reaction contains 50 nM of each primer.

For example, if a particular primer stock is at 100 µM, add 2.5 µl of each primer, up to 200 primers, and nuclease-free water to a total volume of 500 µl. Mix thoroughly, then centrifuge briefly to collect the solution at the bottom of the tube. Store on ice. This will provide enough assay pool for 100 preamplification reactions. Use 5 µl of each assay pool in a 50 µl preamplification reaction. Up to four pools may be added per amplification reaction.

Note: Assay pools are stable at 4ºC for up to 30 days and at –20ºC for up to 1 year.

Prepare Preamplification Reaction Mix

1. Thaw SsoAdvanced PreAmp Supermix to room temperature. Mix thoroughly, then centrifuge briefly to collect the solution at the bottom of the tube. Store on ice.
2. Prepare preamplification reaction mix on ice according to the instructions in Table 1. Good pipetting practice must be employed to ensure assay precision and accuracy.

Table 1. Reaction setup.

Component	Volume in a 50 µl Reaction	Final Concentration
SsoAdvanced PreAmp Supermix (2x)	25 µl	1x
Preamplification assay pool	Variable (see Prepare Preamplification Assay Pool section)	
DNA template*	Variable	cDNA: 250 ng–100 pg Genomic DNA (gDNA): 250 ng–100 pg
Nuclease-free water	Variable	
Total preamplification reaction mix volume	**50 µl**	**–**

* We recommend using cDNA synthesized with Bio-Rad's iScript™ Reverse Transcription Supermix for RT-qPCR (catalog #170-8840) or iScript Advanced cDNA Synthesis Kit for RT-qPCR (#170-8842).

3. Mix the reaction mix thoroughly to ensure homogeneity and dispense into a PCR tube or into the wells of a PCR plate.
4. Program the thermal cycling protocol on a PCR instrument according to Table 2.

Table 2. Thermal cycling protocol.

Thermal Cycler	Polymerase Activation and DNA Denaturation	Denaturation at 95°C	Annealing/ Extension and Plate Read at 58°C	Cycles
Bio-Rad® C1000™, C1000 Touch™, S1000™, T100™ Other thermal cyclers	3 min at 95°C	15 sec	4 min	10–20* Hold at 4°C

* Use 10–12 cycles for ≤100 assays and 12–14 cycles for ≥101 assays. For Fluidigm systems, up to 20 cycles is recommended.

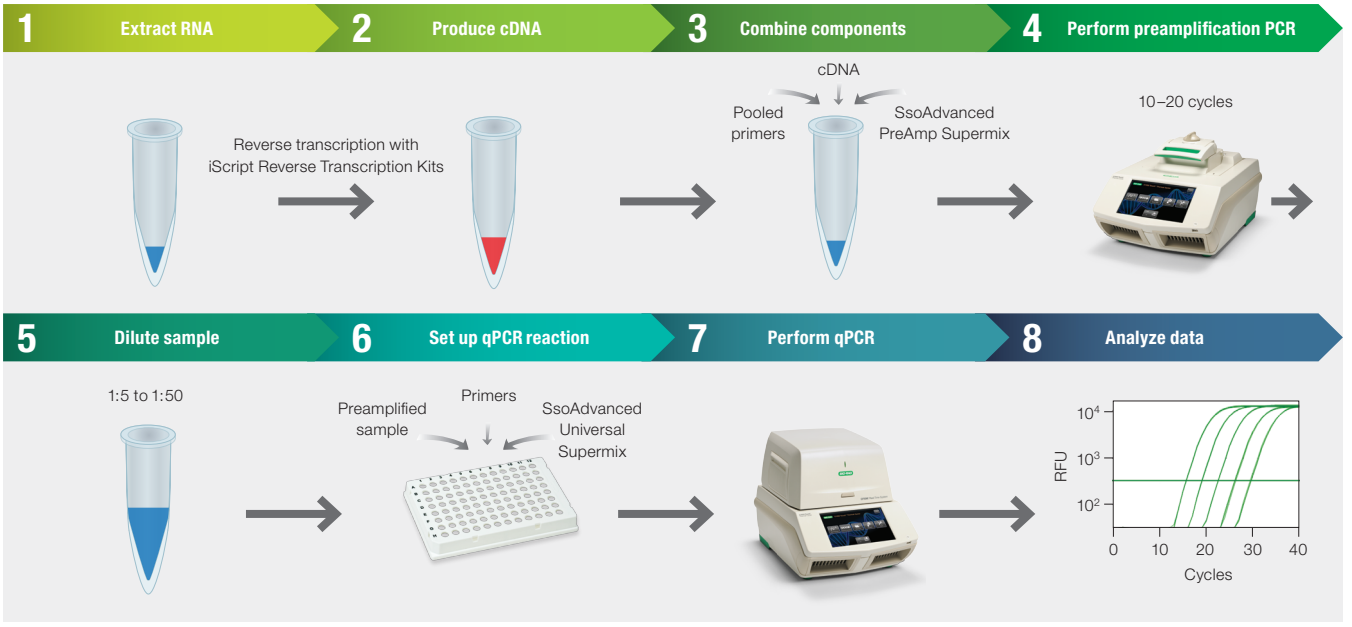

Fig. 1. Preamplification workflow.

5. Load the PCR tubes or plate into a PCR instrument and start the PCR run.
6. After run completion, the preamplification reaction can be stored at –20°C for up to 12 months or at 4°C for up to 72 hr.

Prepare qPCR Reactions

1. The completed preamplification reaction should be diluted a minimum of 1:5 with TE buffer. However, a larger dilution volume may be required depending on the number of assays planned for downstream reverse transcription qPCR and the number of technical replicates. For example, a 1:50 dilution will provide enough volume for 400 qPCR assays using technical triplicates at 2 µl per reaction.
2. Use 2 µl of the dilution per 20 µl qPCR reaction or 1 µl per 10 µl qPCR reaction for a 96- or a 384-well plate, respectively, for optimal results. See Figure 1 for the entire preamplification workflow.

Note: If SsoAdvanced™ Universal SYBR® Green Supermix is used for qPCR of GC-rich targets after preamplification, use 98°C for the activation and denaturation steps for both cDNA and gDNA templates to obtain optimal results.

Quality Control

SsoAdvanced PreAmp Supermix demonstrates high multiplex 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

- PrimePCR PreAmp Assays
- Reverse transcription kits for 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 supermixes:
  - SsoAdvanced™ Universal SYBR® Green Supermix (#172-5270)
  - SsoAdvanced Universal Probes Supermix (#172-5280)

Visit **bio-rad.com/SPS** for more information.

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