iTaq™ SYBR® Green Supermix with ROX

200 x 50 µl reactions	172-5850
500 x 50 µl reactions	172-5851
1,000 x 50 µl reactions	172-5852
2,000 x 50 µl reactions	172-5853

For Research purposes only
Store at -20 °C, protect from light

Storage and Stability

iTaq SYBR Green Supermix with ROX is stable for 1 year when stored in a constant temperature freezer at -20°C, protected from light. For convenience, it may be stored unfrozen at 2–8°C for up to 6 months. After thawing, mix thoroughly before using. Repeated freezing and thawing of the supermix is not recommended.

Kit Contents

iTaq SYBR Green Supermix with ROX 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. Highly specific amplification is crucial to successful qPCR with SYBR Green I technology since this dye binds to and detects any dsDNA generated during amplification. The antibody-mediated hot-start employed by iTaq DNA polymerase sequesters Taq activity prior to the initial PCR denaturation step. Upon heat activation, the antibodies denature irreversibly, releasing fully active Taq DNA polymerase. This enables specific and efficient primer extension with the convenience of room temperature reaction assembly.

The ROX internal reference dye included in the product is used for normalization of fluorescent signal and to correct for well-to-well optical variations in ROX-dependent instrumentation. It allows seamless integration of iTaq SYBR Green Supermix with ROX with ABI Prism™ Sequence Detection Systems.

iTaq SYBR Green Supermix with ROX contains a proprietary buffer and stabilizers specifically optimized for qPCR using SYBR Green I. This supermix provides the highest level of specificity to reduce the occurrence or delay the detection of primer-dimer and other non-specific artifacts.

Reagent	Kit Size	Volume	Description
iTaq SYBR Green Supermix with ROX (Pink Cap)	200 reactions 500 reactions 1,000 reactions 2,000 reactions	1.25 ml x 4 1.25 ml x 10 1.25 ml x 20 50 ml (bottle)	2X reaction buffer, 0.4 mM dATP, 0.4 mM dCTP, 0.4 mM dGTP, iTaq DNA polymerase, 50 units/ml, 6mM M_gCl_2, SYBR Green I dye, 1 µM ROX internal reference dye, and stabilizers.

This product does not contain fluorescein and cannot be used to perform dynamic well factors with the iCycler iQ™ real-time PCR detection system or the MyiQ™ single color real-time PCR detection system. Use of iTaq SYBR Green Supermix with ROX will not interfere with reactions performed on these systems, but does require either an external well factor plate or persistent well factors for data normalization. To use dynamic well factors, we recommend using iQ SYBR Green Supermix (catalog numbers 170-8880 and 170-8882).

If you need extra M_gCl_2, a 50mM M_gCl_2 solution is available free of charge upon request. Please request catalog number 170-8872 for 1.25 ml of this solution.

Quality Control

iTaq SYBR Green Supermix with ROX is free of contaminating DNase and RNase. Functionally, iTaq SYBR Green Supermix with ROX is tested to demonstrate linear resolution over six orders of dynamic range.

Reaction Set Up

Thaw all components at room temperature. Mix vigorously, and then centrifuge to collect contents to the bottom of the tube before using.

Component	Volume per reaction	Final Concentration
iTaq SYBR Green Supermix with ROX	25 µl	1X
Forward primer	Variable	100–500 nM
Reverse primer	Variable	100–500 nM
RNase/DNase-free water	Variable	
DNA template	Variable	
Total Volume	50 µl	

Note: for smaller reaction volumes (*i.e.*, 25µl reactions), scale all components proportionally.

Recommendations for Optimal Results using the iTaq SYBR Green Supermix with ROX:

- Preparation of a reaction cocktail is crucial in quantitative PCR applications to reduce pipetting errors and maximize assay precision and accuracy. Assemble the reaction cocktail with all required components except sample template (genomic DNA or cDNA), and dispense equal aliquots into each reaction tube. Add target sample to each reaction as the final step. Addition of 5 to 10µl of sample volume will improve assay precision. Replicate samples should be assembled as a master mix with a single addition of sample template.
- Suggested input quantities of template are:

  cDNA from 1 pg to 100 ng of total RNA

  100 pg to 100 ng genomic DNA
- Gently mix and ensure that all components are at the bottom of the amplification tube. Centrifuge briefly if needed.
- Full activation of iTaq DNA polymerase occurs within 30 seconds at 95°C. Initial denaturation times greater than 3 minutes are not recommended.
- Suggested cycling conditions:

Initial denaturation:	95°C, 2 to 3 min
PCR cycling (30-45 cycles):	95°C, 10 to 15 sec
	55–60°C, 30 to 45 sec (collect and analyze data)
Melt Curve (dissociation stage):	Refer to instrument instructions (optional)

Reagents and Materials Not Supplied

Gene-specific primers

Pipet tips, aerosol barrier tips, such as:

The Xcluda® Style B, 211-2006

Nuclease-free tubes or plates, such as:

0.2 ml Thin-Wall Tubes, 223-9473 or plates, 223-9441

RNA purification kit, such as:

Aurum™ Total RNA Mini Kit, 732-6820, or

Aurum Total RNA Kit, 2 x 96 well, 732-6800

cDNA Synthesis kits, such as:

iScript™ cDNA Synthesis Kit, 170-8891, or

iScript Select cDNA Synthesis kit, 170-8897

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

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