

iTaq™ Fast SYBR® Green Supermix With ROX

200 x 20 µl reactions	172-5100
500 x 20 µl reactions	172-5101
1,000 x 20 µl reactions	172-5102
2,000 x 20 µl reactions	172-5103

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

Storage and Stability

iTaq fast SYBR Green supermix with ROX is stable for 6 months 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 3 months. After thawing, mix thoroughly before using. Repeated freezing and thawing of the supermix is not recommended.

Kit Contents

iTaq fast 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) on the Applied Biosystems 7500 Fast (or Standard) Real-Time PCR System and Stratagene Mx series real-time PCR systems. The mixture has been optimized to deliver maximum PCR efficiency, sensitivity, and robust fluorescent signal using fast, or conventional, cycling protocols for SYBR Green qPCR. 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 fast SYBR Green supermix with ROX with Applied Biosystems 7500 and Stratagene real-time PCR systems.

iTaq fast 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 fast SYBR Green supermix with ROX (pink cap tubes or clear bottle)	200 reactions	1.0 ml x 2	2X reaction buffer with dNTPs, iTaq DNA polymerase, MgCl ₂ , SYBR Green I dye, ROX internal reference dye, and stabilizers
	500 reactions	1.0 ml x 5	
	1,000 reactions	1.0 ml x 10	
	2,000 reactions	20 ml (bottle)	

The ROX concentration in this mix is optimized for use on the Applied Biosystems 7500 and Stratagene Mx series real-time PCR systems. To utilize this mix on the Applied Biosystems 7900 Real-Time PCR System, additional ROX (catalog number 172-5858) must be added using the following guidelines: 35 µl ROX per 1 ml of iTaq fast SYBR Green supermix with ROX.

This product does not contain fluorescein and cannot be used to perform dynamic well factors with the iCycler iQ™ 5 real-time PCR detection system or the MyiQ™ single color real-time PCR detection system. Use of iTaq fast 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 require extra MgCl₂, a 50 mM MgCl₂ solution is available free of charge upon request. Please request catalog number 170-8872 for 1.25 ml of this solution.

Quality Control

iTaq fast SYBR Green supermix with ROX is free of contaminating DNase and RNase. Functionally, iTaq fast 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, then centrifuge to collect contents to the bottom of the tube before using.

Component	Volume per Reaction	Final Concentration
iTaq fast SYBR Green supermix with ROX	10 µl	1X
Forward primer	Variable	150–900 nM
Reverse primer	Variable	150–900 nM
RNase/DNase-free water	Variable	
DNA template	Variable	
Total Volume	20 µl	

NOTE: For smaller reaction volumes (i.e., 10–15 µl reactions), scale all components proportionally.

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

- Preparation of a reaction cocktail is crucial in qPCR 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 corresponding to 100 fg to 100 ng of total RNA
 - 50 pg to 50 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, 20 sec to 3 min
 - PCR cycling (30–45 cycles) 95°C, 3 sec
 - 55–60°C, 30 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

The Xcluda™ Style B Aerosol Barrier Tips, catalog number 211-2006

Nuclease-free tubes or plates

0.2 ml Thin-Wall Tubes, catalog number TWI-0201

0.2 ml Thin-Wall Plates, catalog numbers HSP-9601 (Low-Profile) or HSS-9601 (Full Height)

RNA purification kits

Aurum™ Total RNA Mini Kit, catalog number 732-6820

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

cDNA Synthesis kits

iScript™ cDNA Synthesis Kit, catalog number 170-8891

iScript Select cDNA Synthesis Kit, catalog number 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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