

## iTaq™ Supermix with ROX

200 x 50 µl reactions	172-5854
500 x 50 µl reactions	172-5855
1,000 x 50 µl reactions	172-5856
2,000 x 50 µl reactions	172-5857

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

### Storage and Stability

iTaq 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 Supermix with ROX is a 2X concentrated, ready-to-use reaction cocktail containing all components, except primers, probe and template for real-time quantitative PCR. The system has been optimized to deliver maximum PCR efficiency, sensitivity and precision. The antibody-mediated hot-start technology employed by iTaq DNA polymerase sequesters polymerase activity prior to the initial PCR denaturation step. Upon heat activation, the antibody denatures irreversibly, releasing fully active iTaq 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 Supermix with ROX with the ABI Prism™ Sequence Detection Systems.

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 Supermix with ROX (Grey cap tubes, or clear bottle)	200 reactions	1.25 ml x 4	2X reaction mixture containing 0.4mM dATP, 0.4mM dCTP, 0.4mM dGTP, 50 U/ml iTaq DNA polymerase, 40 mM Tris-HCl (pH 8.4), 100 mM KCl, 10mM MgCl <sub>2</sub> , 1 µM ROX internal reference dye, and stabilizers.
	500 reactions	1.25 ml x 10	
	1,000 reactions	1.25 ml x 20	
	2,000 reactions	50 ml (bottle)	

If you would like additional MgCl<sub>2</sub>, a 50 mM MgCl<sub>2</sub> solution is available free of charge upon request. Please request catalog number 170-8872 for 1.25 ml of this solution.

### Quality Control

iTaq Supermix with ROX is free of contaminating DNases and RNases. Functionally, iTaq Supermix with ROX is tested to demonstrate linear resolution over at least 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 Supermix with ROX	25 µl	1X
Forward primer	Variable	100–500 nM
Reverse primer	Variable	100–500 nM
Probe	Variable	50–200 nM
RNase/DNase-free water	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 Supermix with ROX:

- Preparation of a reaction cocktail is crucial in quantitative PCR applications to minimize pipeting 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 sample as 5 to 10 µl volumes 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 synthesized from 1 pg to 1 µl of total RNA
  - 100 pg to 1 µg of 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)

## Reagents and Materials Not Supplied

Gene-specific primers and probe

Pipette 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 Kit, such as:

iScript™ cDNA Synthesis Kit, 170-8891  
iScrip 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](http://www.bio-rad.com/amplification)**

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