

## iTaq™ Fast Supermix With ROX

200 x 20 µl reactions	172-5105
500 x 20 µl reactions	172-5106
1,000 x 20 µl reactions	172-5107
2,000 x 20 µl reactions	172-5108

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

### Storage and Stability

iTaq fast supermix with ROX is stable for 12 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 6 months. After thawing, mix thoroughly before using. Repeated freezing and thawing of the supermix is not recommended.

### Kit Contents

iTaq fast 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 (qPCR) on 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, precision, and robust fluorescent signal using fast, or conventional, cycling protocols. 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 fast supermix with ROX with Applied Biosystems 7500 and Stratagene real-time PCR 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 fast supermix with ROX (grey cap tubes or clear bottle)	200 reactions	1.0 ml x 2	2X reaction buffer with dNTPs, iTaq DNA polymerase, MgCl <sub>2</sub> , 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 supermix with ROX.

If you require extra 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 fast supermix with ROX is free of contaminating DNases and RNases. Functionally, iTaq fast 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 fast supermix with ROX	10 µl	1X
Forward primer	Variable	150–900 nM
Reverse primer	Variable	150–900 nM
Probe	Variable	200–250 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 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 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)

## 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](http://www.bio-rad.com/amplification)

## NOTICE TO PURCHASER: LIMITED LICENSE

A license to perform the patented 5' Nuclease Process for research is obtained by the purchase of (i) both Authorized 5' Nuclease Core Kit and Licensed Probe, (ii) a Licensed 5' Nuclease Kit, or (iii) license rights from Applied Biosystems.

This product is an Authorized 5' Nuclease Core Kit. Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,079,352, 5,789,224, 5,618,711, 6,127,155, 5,677,152, (Claims 1-23 only), 5,773,258, (claims 1 and 6 only), 5,407,800, 5,322,770, 5,310,652, 5,210,015, 5,487,972, and claims outside the US corresponding to US Patent No. 4,889,818. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. Separate purchase of a Licensed Probe would convey rights under the applicable claims on US Patents Nos. 5,538,848, 5,723,591, 5,876,930, 6,030,787, 6,258,569, 5,804,375 (claims 1-12 only), and 6,214,979, and corresponding claims outside the United States. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Aurum, iScript, iTaq, and Xcluda are trademarks of Bio-Rad Laboratories. ABI is a trademark of the Applied Biosystems Corporation. Mx series is a trademark of Stratagene, an Agilent Technologies company.