iQ™ Supermix

Catalog #	Supermix Volume	Kit Size
170-8860	2.5 ml (2 x 1.25 ml vials)	100 x 50 µl reactions
170-8862	12.5 ml (10 x 1.25 ml vials)	500 x 50 µl reactions
170-8864	25.0 ml (20 x 1.25 ml vials)	1,000 x 50 µl reactions

For research purposes only.

Storage and Stability
Guaranteed for 12 months in a constant temperature freezer at –20°C. For convenience, this supermix may be stored at 4°C for up to six months. Repeated freezing and thawing of the supermix is not recommended.

Kit Contents
iQ™ supermix is a 2x concentrated, ready-to-use reaction master mix optimized for simplex or duplex probe-based quantitative PCR (qPCR). It contains antibody-mediated hot-start iTaq DNA polymerase, dNTPs, $MgCl_2$, enhancers, and stabilizers.

Instrument Compatibility
This supermix is compatible with all Bio-Rad real-time PCR instruments, and with the Roche LightCycler LC480, QIAGEN Rotor-Gene Q, Eppendorf Mastercycler EP realplex, Stratagene Mx real-time PCR systems (with ROX reference setting turned off), and other ROX-independent real-time PCR instruments.

Reaction Mix Preparation and Thermal Cycling Protocol
1. Thaw iQ™ supermix and other frozen reaction components to room temperature. Mix thoroughly, centrifuge briefly to collect solution at the bottom of tubes, and then store on ice protected from light.

2. Prepare (on ice or at room temperature) enough assay master mix for all reactions by adding all required components except the DNA template according to the following recommendations (Table 1).

Table 1. Reaction Setup*

Component	Volume per 20 µl Reaction	Volume per 10 µl Reaction	Final Concentration
iQ™ supermix (2x)	10 µl	5 µl	1x
Forward and reverse primers	Variable	Variable	100–500 nM each primer
Fluorogenic probe	Variable	Variable	100–500 nM
DNA template	Variable	Variable	cDNA: 100 ng–100 fg Genomic DNA: 500 ng–5 pg
H_2O	Variable	Variable	—
Total reaction mix volume	*20 µl*	*10 µl*	—

* Scale all components proportionally according to sample number and reaction volumes.

3. Mix the assay master mix thoroughly to ensure homogeneity and dispense equal aliquots into each qPCR tube or into the wells of a qPCR plate. Good pipetting practice must be employed to ensure assay precision and accuracy.

4. Add DNA samples (and DNase-free H_2O if needed) to the PCR tubes or wells containing assay master mix (Table 1), seal tubes or wells with flat caps or optically transparent film, and vortex 30 sec or more to ensure thorough mixing of the reaction components. Spin the tubes or plate to remove any air bubbles and collect the reaction mixture in the vessel bottom.

5. Program thermal cycling protocol on the real-time PCR instrument according to Table 2.

6. Load the PCR tubes or plate onto the real-time PCR instrument and start the PCR run.

7. Perform data analysis according to the instrument-specific instructions.

Table 2. Thermal Cycling Protocol					
Real-Time PCR System	**Setting/ Block**	**Polymerase Activation and DNA Denaturation at 95°C**	**Amplification**		
			Denaturation at 95°C	**Annealing/ Extension + Plate Read at an Optimized Temperature**	**Cycles**
Bio-Rad® CFX96™, CFX384™, CFX96 Touch™, CFX384 Touch™, CFX Connect™ systems	Fast	3 min	10–15 sec	30–60 sec*	35–40
Bio-Rad® iQ™5, MiniOpticon™, Chromo-4™, MyiQ™	Standard				
Roche LightCycler 480	Standard				
QIAGEN Rotor-Gene, Stratagene Mx series	Standard				

* Longer annealing/extension time (up to 1 min) should be used for low abundance genes in multiplexing (duplex).

Recommendations for Primer and Probe Design

- For best qPCR efficiency, design assays targeting an amplicon size of 70–150 bp
- The qPCR cycling protocols have been optimized for assays with a primer Tm of 60°C designed using the open source Primer3 program (**http://frodo.wi.mit.edu/**) under its default settings, or using the Primer Express software by Applied Biosystems. For assays designed using other tools, the primer Tm should be recalculated using Primer3 for determining annealing/extension temperature. The probe's Tm must be 10°C higher than the calculated primer Tm

Quality Control

iQ™ supermix demonstrates high 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

- Reverse transcription reagents for 2-step RT-qPCR: iScript™ reverse transcription supermix for RT-qPCR (170-8840), iScript advanced cDNA synthesis kit for RT-qPCR (170-8842), iScript cDNA synthesis kit (170-8890).

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

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