iQ™ SYBR® Green Supermix

Catalog #	Supermix Volume	Kit Size
170-8880	2.5 ml (2 x 1.25 ml vials)	100 x 50 µl reactions
170-8882	12.5 ml (10 x 1.25 ml vials)	500 x 50 µl reactions
170-8884	25.0 ml (20 x 1.25 ml vials)	1,000 x 50 µl reactions
170-8885	50.0 ml (1 x 50 ml bottle)	2,000 x 50 µl reactions
170-8886	25.0 ml (5 x 5 ml vials)	1,000 x 50 µl reactions
170-8887	50.0 ml (10 x 5 ml vials)	2,000 x 50 µl reactions

For research purposes only.

Storage and Stability
Guaranteed for 12 months in a constant temperature freezer at –20°C, protected from light. 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™ SYBR® Green supermix is a 2x concentrated, ready-to-use reaction master mix optimized for dye-based quantitative PCR (qPCR). It contains antibody-mediated hot-start iTaq DNA polymerase, dNTPs, $MgCl_2$, SYBR® Green I dye, enhancers, stabilizers, and fluorescein.

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™ SYBR® Green supermix and other frozen reaction components to room temperature. Mix thoroughly, centrifuge briefly to collect solutions 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 recommendations in Table 1.

Table 1. Reaction Setup*

Component	Volume per 20 µl Reaction	Volume per 10 µl Reaction	Final Concentration
iQ™ SYBR® Green supermix (2x)	10 µl	5 µl	1x
Forward and reverse primers	Variable	Variable	300 nM (100–500 nM each)
DNA template	Variable	Variable	cDNA: 100 ng–100 fg gDNA: 50 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/ Mode	Polymerase Activation and DNA Denaturation at 95°C	Amplification			Melt Curve Analysis
			Denaturation at 95°C	Annealing /Extension + Plate Read at an Optimized Temperature (55–60°C)	Cycles	
Bio-Rad® CFX96™, CFX384™, CFX96 Touch™, CFX384 Touch™, CFX Connect™ systems	SYBR® only	3 min*	10–15 sec	30–60 sec	35–40	55–95°C 0.5°C increment 2–5 sec/step (or use instrument default setting)
Bio-Rad® iQ™5, MiniOpticon™, Chromo4™, MyiQ™	Standard					
Roche LightCycler 480	Standard					
QIAGEN Rotor-Gene and Stratagene Mx series	Standard					

*Genomic DNA templates may need longer denaturation time (up to 5 min).

Recommendations for Optimal Results

- 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. For assays designed using other tools, the primer Tm should be recalculated using Primer3
- To achieve the best performance, carrying out a thermal gradient to determine the optimal annealing/extension temperature is recommended

Quality control

iQ™ SYBR® Green 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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