iTaq™ Universal SYBR® Green Supermix

Catalog #	Supermix Volume	Kit Size
172-5120	2 ml (2 x 1 ml vials)	200 x 20 µl reactions
172-5121	5 ml (5 x 1 ml vials)	500 x 20 µl reactions
172-5122	10 ml (10 x 1 ml vials)	1,000 x 20 µl reactions
172-5124	25 ml (5 x 5 ml vials)	2,500 x 20 µl reactions
172-5125	50 ml (10 x 5 ml vials)	5,000 x 20 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 can be stored at 4°C short-term or refrozen up to ten times.

Kit Contents
iTaq™ Universal SYBR® Green supermix is a 2x concentrated, ready-to-use reaction master mix optimized for dye-based quantitative PCR (qPCR) on any real-time PCR instrument (ROX-independent and ROX-dependent). It contains antibody-mediated hot-start iTaq DNA polymerase, dNTPs, $MgCl_2$, SYBR® Green I dye, enhancers, stabilizers, and a blend of passive reference dyes (including ROX and fluorescein).

Instrument Compatibility
This supermix is compatible with all Bio-Rad and ROX-dependent Applied Biosystems real-time PCR instruments, and with the Roche LightCycler LC480, QIAGEN Rotor-Gene Q, Eppendorf Mastercycler EP realplex, and Stratagene Mx real-time PCR systems.

Reaction Mix Preparation and Thermal Cycling Protocol
1. Thaw iTaq™ Universal 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 following recommendations (Table 1).

Table 1. Reaction Setup*

Component	Volume per 20 µl Reaction	Volume per 10 µl Reaction	Final Concentration
iTaq™ Universal SYBR® Green supermix (2x)	10 µl	5 µl	1x
Forward and reverse primers	Variable	Variable	300–500 nM each
DNA template	Variable	Variable	cDNA: 100 ng–100 fg Genomic DNA: 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 seconds 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 & DNA Denaturation at 95°C	Amplification			Melt-Curve Analysis
			Denaturation at 95°C	Annealing /Extension + Plate Read at 60°C	Cycles	
Bio-Rad® CFX96™, CFX384™, CFX96 Touch™, CFX384 Touch™, CFX Connect™ systems	SYBR® only	20–30 sec for cDNA or 2–5 min for gDNA	2–5 sec	15–30 sec	35–40	65°C–95°C 0.5°C increment 2–5 sec/step (or use instrument default setting)
Bio-Rad® iQ™5, MiniOpticon™, Chromo-4™, MyiQ™	Standard		10–15 sec	15–30 sec		
ABI 7500, StepOne, StepOnePlus, 7900ht, and ViiA7	Fast		1–3 sec	20–30 sec		
	Standard		15 sec	60 sec		
ABI 7300 and 7000	Standard		15 sec	60 sec		
Roche LightCycler 480	Fast		2–5 sec	15–30 sec		
	Standard		15 sec	60 sec		
QIAGEN Rotor-Gene and Stratagene Mx series	Fast		2–5 sec	15–30 sec		

Recommendations for Primer Design

- The iTaq™ Universal SYBR® Green supermix and 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.
- For best qPCR efficiency, design assays targeting an amplicon size of 70–150 bp. For amplicons >250 bp in length or of high GC or AT content, longer annealing/extension times (30–60 sec) can be used.

Quality control

iTaq™ Universal SYBR® Green supermix demonstrates >90% PCR efficiency and linear resolution over seven orders of 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).
- qPCR supermix for probes: iTaq universal probes supermix (172-5130)
- qPCR supermix for HRM: Precision melt supermix (172-5110)

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

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