BIO-RAD

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 up to 3 months.

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 other commercially available 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, 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.
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 the thermal cycling protocol on a real-time PCR instrument according to Table 2.
6. Load the PCR tubes or plate into the real-time PCR instrument and start the PCR run.
7. Perform data analysis according to the instrument-specific instructions.

Table 1. Reaction setup.*

Component	Volume/20 µl Reaction, µl	Volume/10 µl Reaction, µl	Final Concentration
iTaq™ universal SYBR® Green supermix (2x)	10	5	1x
Forward and reverse primers	Variable	Variable	300–500 nM each primer
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.

Table 2. Thermal cycling protocol.

Real-Time PCR System	Setting/ Block	Amplification: Denaturation at 95°C, sec	Amplification: Annealing/Extension and Plate Read at 60°C, sec	Cycles	Melt Curve Analysis	Polymerase Activation and DNA Denaturation at 95°C
Bio-Rad® CFX96™, CFX384™, CFX96 Touch™, CFX96 Touch Deep Well, CFX384 Touch™, CFX Connect™	SYBR® only	2–5	15–30	35–40	65–95°C 0.5°C increments at 2–5 sec/step (or use instrument default setting)	20–30 sec for cDNA or 2–5 min for genomic DNA
Bio-Rad® iQ™5, MiniOpticon™, Chromo4™, MyiQ™	Standard	10–15	15–30			
Applied Biosystems 7500, 7900HT, QuantStudio, StepOne, StepOnePlus, and ViiA 7	Fast	1–3	20–30			
	Standard	15	60			
Applied Biosystems 7300 and 7000	Standard	15	60			
Roche LightCycler 480	Fast	2–5	15–30			
	Standard	15	60			
QIAGEN Rotor-Gene and Stratagene Mx series	Fast	2–5	15–30			

Recommendations for Primer Design

- The iTaq™ universal SYBR® Green supermix and the qPCR cycling protocols have been optimized for assays with a primer melting temperature (T_m) of 60°C and designed using the open source Primer3 program (http://frodo.wi.mit.edu/) under its default settings. For assays designed using other tools, the primer T_m 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 with high GC or AT content, longer annealing/ extension times (30–60 sec) can be used

Quality Control

iTaq™ universal SYBR® Green supermix demonstrates high PCR efficiency and 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 real-time 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)

Reagents for real-time qPCR:

- SsoAdvanced™ universal SYBR® Green supermix (172-5270)
- iTaq™ universal SYBR® Green one-step kit (172-5150)

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

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