BIO-RAD

iTaq™ Universal SYBR® Green One-Step Kit

Catalog #	Reverse Transcriptase Volume	Reaction Mix Volume	Kit Size
172-5150	50 µl (1 x 1 ml vials)	1 ml (1 x 1 ml vials)	100 x 20 µl reactions
172-5151	125 µl (1x 1 ml vials)	5 ml (5 x 1 ml vials)	500 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, the reaction mix tube can be stored at 4°C for up to three months. The reverse transcriptase tube must be stored at –20°C.

Kit Contents
iTaq universal SYBR® Green one-step kit contains iScript™ reverse transcriptase, which is an RNase H+ MMLV enzyme engineered to deliver uncompromised sensitivity and true representation of target RNA level, plus a potent blend of RNase inhibitors and our patented RT inhibition reducer that prevents RNA degradation and mispriming during reaction setup and reverse transcription to ensure optimal RT efficiency.

The reaction mix is 2x concentrated and optimized for SYBR® Green-based, one-step real-time PCR on any real-time PCR instrument (ROX-independent and ROX-dependent). It contains antibody-mediated hot-start iTaq DNA polymerase, dNTPs, Mg^{++}, enhancers, stabilizers, and a blend of passive reference dyes (including ROX).

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 reaction mix and other frozen reaction components to 4°C. Mix thoroughly, centrifuge briefly to collect solutions at the bottom of tubes, and then store on ice protected from light.

2. Prepare on ice enough reaction setup for all reactions by adding all required components *except* RNA 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 reaction mix (2x)	10 µl	5 µl	1x
iScript reverse transcriptase	0.25 µl	0.125 µl	1x
Forward and reverse primers	Variable	Variable	300 nM** each
RNA **(add at step 4)**	Variable	Variable	RNA: Up to 500 ng
Nuclease-free H_2O	Variable	Variable	—
Total reaction mix volume	*20 µl*	*10 µl*	—

* Scale all components proportionally according to sample number and reaction volumes.
** To validate the optimal primer concentration, perform a primer matrix to determine final primer concentration.

3. Mix the reaction setup thoroughly to ensure homogeneity and dispense equal aliquots into each PCR tube or into the wells of a PCR plate. Use good pipetting practice to ensure assay precision and accuracy.

4. Add RNA (and nuclease-free H_2O, if needed) to the PCR tubes or wells containing the reaction setup (Table 1), seal tubes or wells with flat caps or optically transparent film, and gently vortex 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.

Table 2. Thermal Cycling Protocol

Real-Time PCR System	Setting/ Scan Mode	Reverse Transcription Reaction	Polymerase Activation and DNA Denaturation	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®/FAM	10 min at **50°C**	1 min at **95°C**	10 sec	10–30 sec	35–40	65–95°C 0.5°C increment 2–5 sec/step (or use instrument default setting)
Bio-Rad® iQ™5, MiniOpticon™, Chromo4™, MyiQ™	Standard				15–30 sec		
AB 7500, StepOne, StepOnePlus, 7900HT, ViiA7	Standard			15 Sec	60 sec		
Roche LightCycler 480	Fast				10–30 sec		
	Standard				60 sec		
Qiagen Rotor-Gene and Stratagene Mx series	Fast				30 sec		

** Shorter annealing/extension times (1–10 sec) can be used for amplicons <100 bp. Longer annealing/extension times (30–60 sec or more) can be used for amplicons >250 bp, GC- or AT- rich targets, and crude samples, or for higher input amounts (for example, >100 ng of RNA).

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

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

Recommendations for Assay Design and Optimization

- For best qPCR efficiency, design assays targeting an amplicon size of 70–150 bp
- The iTaq universal SYBR® Green one-step kit cycling protocols have been optimized for assays with a primer melting temperature (T_m) of 60°C that were designed using the open source Primer3, Primer3Plus, or Primer-BLAST programs under default settings. If primers are designed using other programs, adjust the temperature accordingly

Quality Control

iTaq universal SYBR® Green one-step kit demonstrates high RT-qPCR 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 two-step real-time PCR:
  - iScript advanced cDNA synthesis kit for RT-qPCR (170-8842)
  - iScript reverse transcription supermix for RT-qPCR (170-8840)
- Real-time PCR supermixes for SYBR® Green-based qPCR:
  - SsoAdvanced™ universal SYBR® Green supermix (172-5270)
  - iTaq universal SYBR® Green supermix (172-5120)

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

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