

iTaq™ Universal Probes Supermix

Catalog #	Supermix Volume	Kit Size
172-5130	2 ml (2 x 1 ml vials)	200 x 20 µl reactions
172-5131	5 ml (5 x 1 ml vials)	500 x 20 µl reactions
172-5132	10 ml (10 x 1 ml vials)	1,000 x 20 µl reactions
172-5134	25 ml (5 x 5 ml vials)	2,500 x 20 µl reactions
172-5135	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 probes supermix is a 2x concentrated, ready-to-use reaction master mix containing all components, except primers, probes, and template, for probe-based real-time quantitative PCR (qPCR; simplex or duplex) on any real-time instrument (ROX-independent and ROX-dependent). It contains antibody-mediated hot-start iTaq DNA polymerase, dNTPs, MgCl₂, enhancers, stabilizers, and a blend of passive reference dyes (including ROX).

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 probes 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 H₂O 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 probes supermix (2x)	10	5	1x
Forward and reverse primers	Variable	Variable	150–900 nM each primer**
Fluorogenic probe	Variable	Variable	150–250 nM each probe
DNA template	Variable	Variable	cDNA: 100 ng–100 fg Genomic DNA: 500 ng–5 pg
H ₂ O	Variable	Variable	—
Total reaction mix volume	20 µl	10 µl	—

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

** For duplex assays with large copy number differences, decreasing the primer concentration of the higher-copy target to 150 nM and increasing low-copy target primers to 900 nM will help achieve optimal results.

Table 2. Thermal cycling protocol.

Real-Time PCR System	Setting/Block	Amplification			Polymerase Activation and DNA Denaturation at 95°C
		Denaturation at 95°C, sec	Annealing/Extension and Plate Read at 60°C, sec*	Cycles	
Bio-Rad [®] CFX96 [™] , CFX384 [™] , CFX96 Touch [™] , CFX96 Touch Deep Well, CFX384 Touch [™] , CFX Connect [™]	Fast	2–5	15–30	35–40	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	20		
	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		

* Longer annealing/extension time for low-abundance targets (up to 1 min) may be optimal for multiplexing (instrument dependent).

Recommendations for Primer and Probe Design

- The iQ[™] universal probes 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. The probe T_m must be 10°C higher than the calculated primer T_m .
- 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

iQ[™] universal probes 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 probes supermix (172-5280)
- iQ[™] universal probes one-step kit (172-5140)

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

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