

iTaq Universal Probes One-Step Kit

Catalog #	Description
1725140	iTaq Universal Probes One-Step Kit, 100 x 20 µl reactions
1725141	iTaq Universal Probes One-Step Kit, 500 x 20 µl reactions
12013250	iTaq Universal Probes One-Step Kit, 5,000 x 20 µl reactions

For research purposes only.

Introduction

iTaq Universal Probes One-Step Kit is an easy two-tube system optimized for probe-based, singleplex, or duplex reverse transcription PCR on any real-time PCR system. iScript Reverse Transcriptase is an RNase H+ Moloney murine leukemia virus (MMLV) enzyme engineered to deliver uncompromised sensitivity and true representation of target RNA level. A potent blend of RNase inhibitors prevents RNA degradation during reaction setup and reverse transcription. iTaq Universal Probes Reaction Mix is a 2x concentrated, ready-to-use reaction mix containing antibody-mediated hot-start Taq DNA polymerase, dNTPs, MgCl₂, enhancers, stabilizers, and universal reference dyes. See Table 1 for kit contents.

Table 1. Kit contents.

Catalog #	2x iTaq Universal Probes Reaction Mix	iScript Reverse Transcriptase	Nuclease-Free Water
1725140	1 ml (1 x 1 ml vial)	50 µl (1 x 50 µl vial)	1.5 ml (1 x 1.5 ml vial)
1725141	5 ml (5 x 1 ml vials)	250 µl (2 x 125 µl vials)	4.5 ml (3 x 1.5 ml vials)
12013250	50 ml (1 x 50 ml bottle)	2.5 ml (1 x 2.5 ml vial)	45 ml (1 x 45 ml bottle)

Storage and Stability

Guaranteed for 12 months if stored 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 3 months. The reverse transcriptase tube must be stored at -20°C.

Instrument Compatibility

This supermix is compatible with all Bio-Rad and ROX-dependent Applied Biosystems real-time PCR instruments, and with the Roche LightCycler 480, 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 Probes Reaction Mix and other frozen reaction components to 4°C. Mix thoroughly, centrifuge briefly to collect solution at the bottom of tubes, and then store on ice protected from light.
2. Prepare on ice enough reaction mix for all reactions by adding all required components, except RNA, according to the following recommendations (Table 2).

Table 2. Reaction setup.*

Component	Volume per 20 µl Reaction	Volume per 10 µl Reaction	Final Concentration
2x iTaq Universal Probes Reaction Mix	10 µl	5 µl	1x
iScript Reverse Transcriptase	0.5 µl	0.25 µl	1x
Forward and reverse primers	Variable	Variable	100–900 nM** each
Fluorogenic probe(s)	Variable	Variable	150–250 nM each
RNA (add at step 4)	Variable	Variable	RNA: 100 ng–100 fg
Nuclease-free water	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 ΔC_q (ΔC_q) values, decreasing the primer concentrations for the higher expressing target may help. To validate, perform a primer matrix to determine optimal final primer concentration.

3. Mix the reaction mix 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 water, if needed) to the PCR tubes or wells containing the reaction setup (Table 2), 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 3.

Table 3. Thermal cycling protocol.

Real-Time PCR System	Setting/Scan Mode	Reverse Transcription Reaction	Polymerase Activation and DNA Denaturation	Amplification		
				Denaturation at 95°C	Annealing/Extension + Plate Read at 60°C, sec*	Cycles
Bio-Rad CFX96, CFX384, CFX96 Touch, CFX384 Touch, CFX Connect	All channels	10 min at 50°C	1–3 min at 95°C	2–15 sec	10–30	35–40
Bio-Rad iQ5, MiniOpticon, Chromo4, MyiQ	Standard				15–30	
Applied Biosystems 7500, StepOne, StepOnePlus, 7900HT, QuantStudio, and ViiA 7	Fast				10–30	
	Standard				60	
	Fast				10–30	
Roche LightCycler 480	Standard				60	
QIAGEN Rotor-Gene and Stratagene Mx series	Fast	10–30				

* Shorter annealing/extension times (5–10 sec) may be used for amplicons <100 bp. Longer annealing/extension times (30–60 sec or more) may be used for amplicons >250 bp, GC- or AT-rich targets, crude samples, or for higher input amounts (for example, ≥100 ng of RNA).

- Load the PCR tubes or plate onto the real-time PCR instrument and start the RT-qPCR run.
- 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 iQ Universal Probes 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
- The probe's T_m should be 8–10°C higher than the calculated primer T_m . In a duplex reaction, applying the brighter fluorophores to the lower-expressing targets and the less bright fluorophores to the higher-expressing targets can help in visualizing data

Quality Control

iQ Universal Probes One-Step Kit demonstrates high RT-qPCR efficiency and linear resolution over a wide linear dynamic range. iQ Universal Probes One-Step Kit is manufactured under ISO 13485:2016 to ensure lot-to-lot consistency. This product is free of detectable DNase and RNase activities.

Related Products

Catalog #	Description
12010176	Reliance One-Step Multiplex RT-qPCR Supermix , 200 x 20 µl reactions
1725150	iQ Universal SYBR® Green One-Step Kit , 100 x 20 µl reactions
1725080	SingleShot Cell Lysis Kit , 100 x 50 µl reactions
1725081	SingleShot Cell Lysis Kit , 500 x 50 µl reactions

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

Visit bio-rad.com/PrimePCR for expertly predesigned probe assays for gene expression.

Visit bio-rad.com/PCRPlastics for a full range of PCR plastics and seals.

Visit bio-rad.com/OneStepRTqPCR for more information.

BIO-RAD is a trademark of Bio-Rad Laboratories, Inc.

All trademarks used herein are the property of their respective owner.

SYBR is a trademark of Thermo Fisher Scientific Inc. Bio-Rad Laboratories, Inc. is licensed by Thermo Fisher Scientific Inc to sell reagents containing SYBR Green I for use in real-time PCR, for research purposes only.