# iTaq™ Universal Probes One-Step Kit

| Catalog # | Reverse Transcriptase Volume | Reaction Mix Volume | Kit Size |
|---|---|---|---|
| 172-5140 | 50 µl (1 x 50 µl vials) | 1 ml (1 x 1 ml vials) | 100 x 20 µl reactions |
| 172-5141 | 250 µl (2 x 125 µl 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 probes one-step kit contains iScript™ advanced reverse transcriptase, an RNase H+ 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.

The reaction mix is a 2x concentrated, ready-to-use reaction mix optimized for probe-based, one-step (singleplex and duplex) real-time PCR on any real-time PCR instrument (ROX-independent and ROX-dependent). It contains antibody-mediated hot-start Taq DNA polymerase, dNTPs, $MgCl_2$, 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 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 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 probes reaction mix (2x) | 10 µl | 5 µl | 1x |
| iScript advanced 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 $H_2O$ | Variable | Variable | — |
| *Total reaction setup volume* | *20 µl* | *10 µl* | — |

* Scale all components proportionally according to sample number and reaction volumes.
** For duplex assays with large ΔCq ($\Delta C_T$) 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 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 | | |
|---|---|---|---|---|---|---|
| | | | | Denaturation at 95°C | Annealing/ Extension + Plate Read at 60°C** | Cycles |
| Bio-Rad® CFX96™, CFX384™, CFX96 Touch™, CFX384 Touch™, CFX Connect™ systems | All channels | 10 min at **50°C** | 1-3 min at **95°C** | 2–15 sec | 10–30 sec | 35–40 |
| Bio-Rad® iQ™5, MiniOpticon™, Chromo4™, MyiQ™ | Standard | | | | 15–30 sec | |
| AB 7500, StepOne, StepOnePlus, 7900HT, and ViiA 7 | Fast | | | | 10–30 sec | |
| | Standard | | | | 60 sec | |
| Roche LightCycler 480 | Fast | | | | 10–30 sec | |
| | Standard | | | | 60 sec | |
| Qiagen Rotor-Gene and Stratagene Mx series | Fast | | | | 10–30 sec | |

** 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).

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 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**

iTaq universal probes 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**

- One-step real-time PCR kits for SYBR® Green-based RT-qPCR:
  - iTaq™ Universal SYBR® Green One-Step Kit (172-5150)
- 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 probe-based qPCR:
  - SsoAdvanced™ universal probes supermix (172-5180)
  - iTaq™ universal probes supermix (172-5130)

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



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