**iTaquer Universal Probes Supermix**

**Catalog #**

- 1725130 iTaq Universal Probes Supermix, 2 ml (2 x 1 ml vials), 200 x 20 µl reactions
- 1725131 iTaq Universal Probes Supermix, 5 ml (5 x 1 ml vials), 500 x 20 µl reactions
- 1725132 iTaq Universal Probes Supermix, 10 ml (10 x 1 ml vials), 1,000 x 20 µl reactions
- 1725134 iTaq Universal Probes Supermix, 25 ml (5 x 5 ml vials), 2,500 x 20 µl reactions
- 1725135 iTaq Universal Probes Supermix, 50 ml (10 x 5 ml vials), 5,000 x 20 µl reactions

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**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 for up to 3 months.

**Kit Contents**

iTaquer 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 PCR 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, centrifug e 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 reaction mix for all qPCR reactions by adding all required components, except the DNA template, according to the recommendations in Table 1.

3. Mix the reaction 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 nuclease-free H₂O if needed) to the PCR tubes or wells containing reaction 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.

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**Table 1. Reaction setup.**

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume/20 µl Reaction, µl</th>
<th>Volume/10 µl Reaction, µl</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>iTaq Universal Probes Supermix (2x)</td>
<td>10</td>
<td>5</td>
<td>1x</td>
</tr>
<tr>
<td>Forward and reverse primers</td>
<td>Variable</td>
<td>Variable</td>
<td>150–900 nM each primer**</td>
</tr>
<tr>
<td>Fluorogenic probe</td>
<td>Variable</td>
<td>Variable</td>
<td>150–250 nM each probe</td>
</tr>
<tr>
<td>DNA template (add at step 4)</td>
<td>Variable</td>
<td>Variable</td>
<td>cDNA: 100 ng–100 fg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Genomic DNA: 50 ng–5 pg</td>
</tr>
<tr>
<td>Nuclease-free H₂O</td>
<td>To 20 µl</td>
<td>To 10 µl</td>
<td>—</td>
</tr>
<tr>
<td>Total reaction mix volume</td>
<td>20 µl</td>
<td>10 µl</td>
<td>—</td>
</tr>
</tbody>
</table>

* 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 achieves optimal results.
Table 2. Thermal cycling protocol.

<table>
<thead>
<tr>
<th>Real-Time PCR System</th>
<th>Setting/Block</th>
<th>Polymerase Activation and DNA Denaturation at 95°C</th>
<th>Denaturation at 95°C, sec</th>
<th>Annealing/Extension and Plate Read at 60°C, sec*</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Rad CFX96, CFX384, CFX96 Touch, CFX96 Touch Deep Well, CFX384-Touch, CFX Connect</td>
<td>Fast</td>
<td>20–30 sec for cDNA or 2–5 min for genomic DNA</td>
<td>2–5</td>
<td>15–30</td>
<td>35–40</td>
</tr>
<tr>
<td>Bio-Rad Chromo4, IQ5, MiniOpticon, MyIQ</td>
<td>Standard</td>
<td></td>
<td>10–15</td>
<td>15–30</td>
<td></td>
</tr>
<tr>
<td>Applied Biosystems 7500, 7900HT, QuantStudio, StepOne, StepOnePlus, and VIA 7</td>
<td>Standard</td>
<td></td>
<td>1</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Applied Biosystems 7000 and 7300</td>
<td>Standard</td>
<td></td>
<td>15</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Roche LightCycler 480</td>
<td>Fast</td>
<td></td>
<td>15</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>QIAGEN Rotor-Gene and Stratagene Mx series</td>
<td>Standard</td>
<td></td>
<td>2–5</td>
<td>15–30</td>
<td></td>
</tr>
</tbody>
</table>

* 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 iTaq 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/](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

iTaq Universal Probes Supermix demonstrates high PCR efficiency and a wide dynamic range. It is manufactured under ISO 13485:2016 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 (1708840)
- iScript Advanced cDNA Synthesis Kit for RT-qPCR (1725037)
- iScript qDNA Clear cDNA Synthesis Kit (1725034)
- iScript cDNA Synthesis Kit (1708890)

Reagents for real-time qPCR:
- SsoAdvanced Universal Probes Supermix (1725280)
- iTaq Universal Probes One-Step Kit (1725140)

PCR primer and probe assays for real-time qPCR:
- PrimePCR Assays and Panels

Visit [bio-rad.com/amplification](http://bio-rad.com/amplification) to learn more about Bio-Rad’s complete solution for amplification.

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