USB® Taq PCR Master Mix (2X)
Product number 71162/3

Brief protocol

USB Taq PCR Master Mix is supplied in a convenient 2X pre-mixed formulation containing PCR-qualified Taq DNA Polymerase, nucleotides, and reaction buffer optimized for a wide variety of PCR applications. Taq PCR Master Mix saves time and provides for more consistent performance. The following describes the recommended use of Taq PCR Master Mix.

1. Thaw Taq PCR Master Mix at room temperature. Mix thoroughly and spin briefly in a microcentrifuge to collect tube contents. Place mix on ice.

2. Assemble reaction tubes on ice whenever possible to avoid premature, non-specific polymerase activity.

3. The following is a table of recommended volumes required for different size PCR reactions.

<table>
<thead>
<tr>
<th>Volume</th>
<th>Taq PCR Master Mix</th>
<th>10 µM Forward Primer</th>
<th>10 µM Reverse Primer</th>
<th>DNA Template</th>
<th>Nuclease-Free H₂O up to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Conc.</td>
<td>1X</td>
<td>0.1-1.0 µM</td>
<td>0.1-1.0 µM</td>
<td>&lt; 500 ng</td>
<td>N.A.</td>
</tr>
<tr>
<td>12.5 µl</td>
<td>25 µl</td>
<td>25 µl</td>
<td>25 µl</td>
<td>50 µl</td>
<td></td>
</tr>
<tr>
<td>25 µl</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
<td>100 µl</td>
<td></td>
</tr>
</tbody>
</table>

4. Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.

5. Optional—Overlay reactions with one-half volume PCR-grade mineral oil (PN 71600) when not using heated lid on thermal cycler.

Note: Aerosol-resistant barrier tips and PCR-dedicated components are recommended to avoid contamination of PCR reactions. Use of PCR-grade thin-walled tubes are also recommended.

6. Optional—Additional MgCl₂ may be used to optimize reactions.

7. General cycling guidelines:
   - Initial Denaturation: 95°C for 1-2 minutes
   - Perform 25-35* cycles as follows:
     - Denature: 95°C for 30 seconds
     - Anneal**: 55°C for 30 seconds
     - Extend***: 72°C for 1 minute
     - Final Extension: 72°C for 3 minutes
     - Final Soak: 4-10°C
   - * 45 cycles may be required for low-copy targets.
   - ** Initially, annealing temperature should be 5°C below the calculated Tₘ of the primers. If non-specific products are produced, increase the temperature in 1-2°C increments.
   - *** For primer extension, 72°C is recommended, although the useful range is between 68-74°C. Extension time should be about one minute for every 1 kb of expected product size.

Note: Placing reaction tubes in a pre-warmed cycler set at 80°C may help amplify low-copy or difficult targets.

Colonial PCR:
- Prepare reactions as outlined in step 3 and for DNA template, add one small bacterial colony (< 1 mm) into mix with pipetting motion.
- Perform cycle profile as normal but increase time of initial denaturation step at 95°C to 5 minutes.

Long-PCR (up to 8 kb):
- Prepare reactions up to step 4.
- Reduce extension temperature to 68°C throughout cycling profile. Also, ensure that extension time is suited to product size (i.e., 1 minute/kb) and include auto-extend of 5 seconds/cycle if cycler is equipped with that feature.

For research use only. Not for use in diagnostic procedures.

Affymetrix and USB are registered trademarks of Affymetrix, Inc.

Tag DNA Polymerase—sold under licensing arrangements with Applied Biosystems. Purchase is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) process in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased, i.e., an authorized thermal cycler.

© 2014 Affymetrix, Inc. All rights reserved.

Affymetrix, Inc. usa.affymetrix.com
USA: USBtechsupport@affymetrix.com
Europe: USBtechsupporteurope@affymetrix.com
P 71162B
rev 06/14