**Takyon™ Rox Probe MasterMix dTTP**

**UF-RPMT-C0101 • UF-RPMT-C0701 • UF-RPMT-C0705 • UF-RPMT-C0710**

**[1.5 mL] • [7.5 mL] • [5 x 7.5 mL] • [10 x 7.5 mL]**

Emerging from the combination of an optimized reaction buffer and the new efficient «Takyon™» enzyme, Takyon™ kits for Probe Assays ensure sensitivity and fast delivery of accurate and reproducible results!

**Storage conditions**

For long term storage the Takyon™ Rox Probe MasterMix dTTP should be stored at a temperature between -15 °C and -25 °C in a constant temperature freezer. When stored under these conditions, the components are stable for 24 months. For short term storage the Takyon™ Rox Probe MasterMix dTTP can be stored at 4 °C for 6 months.

**Kit contents** (Table 1)
The kit UF-RPMT-C0701 (UF-RPMT-C0101) contains enough reagents for up to 750 (150) - 20 μL reactions using the performant hotstart Takyon™ enzyme.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Description</th>
</tr>
</thead>
</table>
| 2x MasterMix (blue cap) | 1.5 ml | One tube/bottle of 2x reaction mix containing e.g.:  
- Takyon™ DNA polymerase,  
- MgCl₂ (5.5 mM final concentration),  
- dNTPs,  
- Rox Passive reference,  
- Stabilizers. |
| | 7.5 ml |  
| 50 mM MgCl₂ (clear cap) | 1.5 ml | 50 mM MgCl₂ solution (optional use) |

**Procedure**

1- Thaw all required reagents completely and put them on ice. Mix all reagents well by inversion and spin them down prior to pipetting.

2- Prepare the reaction mix (see Table 2). To correct for dispensing losses, prepare an excess of reaction mix (e.g. a 100-reaction mix for 96 reactions).

3- Add all components together, except for the template. Mix thoroughly by pipetting or inversion. Spin down.

4- Pipette either 2.5 μL of the template cDNA/DNA for your samples or 2.5 μL of the control DNA for your positive control or 2.5 μL of water/buffer for your negative control into your qPCR tubes / plate.

5- Add 17.5 μL of the reaction mix per well / vial, close the plate / vial and mix gently on a stirrer and spin down. Ensure that no bubbles are present in the reaction wells / vials. Reaction set up can be done at room temperature.

6- The Takyon™ Rox Probe MasterMix dTTP will produce consistent and sensitive results under FAST and REGULAR cycling conditions. Program the Real-Time thermocycler using the following recommended parameters (Table 3):

### Table 3

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (μL)</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takyon™ MasterMix</td>
<td>10</td>
<td>1x</td>
</tr>
<tr>
<td>Forward primer</td>
<td>2</td>
<td>50-900 nM¹</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>2</td>
<td>50-900 nM¹</td>
</tr>
<tr>
<td>Probe</td>
<td>2</td>
<td>100-250 nM²</td>
</tr>
<tr>
<td>Water</td>
<td>1.5</td>
<td>(volume is 20 μL minus all other components)²</td>
</tr>
<tr>
<td>Total Mix / reaction</td>
<td>17.5 μL</td>
<td>²</td>
</tr>
</tbody>
</table>

---

**Note 1:** Primer and probe concentrations of 300 nM & 250 nM, respectively, are recommended as starting concentrations. These concentrations will be correct for many assays, but additional optimization of the primer concentrations and primer-probe ratio may be required to obtain the best results with your primer-probe set (see table 4).

**Note 2:** 17.5 μL of reaction mix is added to 2.5 μL of template/control DNA prior to cycling, giving a final reaction volume of 20 μL. See steps 4 and 5. These volumes, including primers & probes, can be adjusted depending on the template and reaction volumes.

---

**Storage conditions**

**For long term storage the Takyon™ Rox Probe MasterMix dTTP should be stored at a temperature between -15 °C and -25 °C in a constant temperature freezer. When stored under these conditions, the components are stable for 24 months. For short term storage the Takyon™ Rox Probe MasterMix dTTP can be stored at 4 °C for 6 months.**

**Kit contents** (Table 1)

The kit UF-RPMT-C0701 (UF-RPMT-C0101) contains enough reagents for up to 750 (150) - 20 μL reactions using the performant hotstart Takyon™ enzyme.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Description</th>
</tr>
</thead>
</table>
| 2x MasterMix (blue cap) | 1.5 ml | One tube/bottle of 2x reaction mix containing e.g.:  
- Takyon™ DNA polymerase,  
- MgCl₂ (5.5 mM final concentration),  
- dNTPs,  
- Rox Passive reference,  
- Stabilizers. |
| | 7.5 ml |  
| 50 mM MgCl₂ (clear cap) | 1.5 ml | 50 mM MgCl₂ solution (optional use) |

**Procedure**

1- Thaw all required reagents completely and put them on ice. Mix all reagents well by inversion and spin them down prior to pipetting.

2- Prepare the reaction mix (see Table 2). To correct for dispensing losses, prepare an excess of reaction mix (e.g. a 100-reaction mix for 96 reactions).

3- Add all components together, except for the template. Mix thoroughly by pipetting or inversion. Spin down.

4- Pipette either 2.5 μL of the template cDNA/DNA for your samples or 2.5 μL of the control DNA for your positive control or 2.5 μL of water/buffer for your negative control into your qPCR tubes / plate.

5- Add 17.5 μL of the reaction mix per well / vial, close the plate / vial and mix gently on a stirrer and spin down. Ensure that no bubbles are present in the reaction wells / vials. Reaction set up can be done at room temperature.

6- The Takyon™ Rox Probe MasterMix dTTP will produce consistent and sensitive results under FAST and REGULAR cycling conditions. Program the Real-Time thermocycler using the following recommended parameters (Table 3):

### Table 3

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (μL)</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takyon™ MasterMix</td>
<td>10</td>
<td>1x</td>
</tr>
<tr>
<td>Forward primer</td>
<td>2</td>
<td>50-900 nM¹</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>2</td>
<td>50-900 nM¹</td>
</tr>
<tr>
<td>Probe</td>
<td>2</td>
<td>100-250 nM²</td>
</tr>
<tr>
<td>Water</td>
<td>1.5</td>
<td>(volume is 20 μL minus all other components)²</td>
</tr>
<tr>
<td>Total Mix / reaction</td>
<td>17.5 μL</td>
<td>²</td>
</tr>
</tbody>
</table>

---

**Note 1:** Primer and probe concentrations of 300 nM & 250 nM, respectively, are recommended as starting concentrations. These concentrations will be correct for many assays, but additional optimization of the primer concentrations and primer-probe ratio may be required to obtain the best results with your primer-probe set (see table 4).

**Note 2:** 17.5 μL of reaction mix is added to 2.5 μL of template/control DNA prior to cycling, giving a final reaction volume of 20 μL. See steps 4 and 5. These volumes, including primers & probes, can be adjusted depending on the template and reaction volumes.
Technical information

Primer and probe design guidelines

Probes:
- Avoid runs of identical nucleotides, especially of 4 or more Gs.
- The probe Tm should be 7 to 10 °C above primers Tm.
- Avoid 5’-end G as it quenches the fluorophore.
- For genotyping, the position of the polymorphism should be in the centre of the probes, and the probe length should be adjusted such that each probe has the same Tm.

Primers:
- GC content should be between 30 % and 80 % (ideally 40-60 %).
- Avoid runs of identical nucleotides, especially of 3 or more Gs or Cs at the 3’ end.
- The Tm should be between 58 °C and 60 °C.
- The primer should be placed as close as possible to the probe.

Custom assay design

The commonly used concentrations for primers and for probes are 300 nM and 100 nM respectively. Optimal results may require titration of primers and probes or adjustment of the primer / probe ratio. The purpose of such a process is to determine the minimum amount of primers and probe required to obtain the most sensitive results with your assay.

Primer titration matrix

Titrates according to the Table 4, perform qPCR and select the concentration which gives the lowest Cq value. By doing this type of titration it is also possible to compensate for differences up to 2 °C in melt temperature of the primers.

Table 4: Primer titration matrix

<table>
<thead>
<tr>
<th>Reverse</th>
<th>50 nM</th>
<th>300 nM</th>
<th>900 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 nM</td>
<td>50 / 50</td>
<td>300 / 50</td>
<td>900 / 50</td>
</tr>
<tr>
<td>300 nM</td>
<td>50 / 300</td>
<td>300 / 300</td>
<td>900 / 300</td>
</tr>
<tr>
<td>900 nM</td>
<td>50 / 900</td>
<td>300 / 900</td>
<td>900 / 900</td>
</tr>
</tbody>
</table>

For further information please contact our Customer Help Desk:

For Europe:
E-mail: info@eurogentec.com
Tel: +32 4 372 76 65 • Toll-free: + 800 666 00 123

For USA:
E-mail: service@anaspec.com
Tel.: +1-510-791-9560 • Toll-free: +1-800-452-5530

Primer-probe ratio matrix

Select optimal primer concentration as described in Table 4 and test with all probe concentrations described in Table 5. Select the concentration which gives the lowest Cq value.

Table 5: Primer-probe ratio matrix

<table>
<thead>
<tr>
<th>Opt. primers conc.</th>
<th>50 nM</th>
<th>100 nM</th>
<th>250 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MgCl₂ adjustment matrix

Standard MgCl₂ concentration is 5.5 mM but optimal MgCl₂ concentration can vary between assays. If necessary adjust the MgCl₂ concentration with the provided 50 mM MgCl₂ tube. Always prefer optimizing the primer and probe concentrations before the MgCl₂ concentration. Adjust the amount of water if MgCl₂ is added to the reaction.