

## Product Data Sheet

*Research use only; not to be administered to humans or used for medical diagnostics*

### Takyon™ Dry Rox One-Step RT Probe MasterMix

UFD-RPRT-C0101 • UFD-RPRT-C0100

3 x 50 (20 µL) RXN • 1 x 50 (20 µL) RXN

#### Storage condition

The Takyon™ Dry Rox One-Step RT Probe MasterMix can be stored at ambient temperature (15-35°C) up to 18-months.\*

After resuspension the Takyon™ Dry Rox One-Step RT Probe MasterMix may be stored as per follow (Table1):

Format		Temperature	Validity period
Dried		15-35°C	18 months*
Liquid	in PCR grade water	4°C	Use upon resuspension
		-20°C	
	in Buffer S	4°C	24 hours
		-20°C	1 month

Table 1: storage condition

\* for unopened bottles (inert gas inside for long term stability)

!!! Do not expose the dried reagent to light as ROX normalisation dye is light sensitive.

#### Kit Content

The Takyon™ Dry Rox One-Step RT Probe MasterMix contains enough reagents for up to 150 (3x50 RXN) - 20 µL reactions using the performant HotStart Takyon™ enzyme.

Reagent	Volume	Description
MasterMix (Lyophilised)	3 x 50 RXN (20 µL) UFD-RPRT-C0101  1 x 50 (20 µL) RXN UFD-RPRT-C0100	1 bottle of MasterMix (50 RXN) contains e.g.: – Takyon™ DNA polymerase, – Euroscript II RT, – RNase Inhibitor, – dNTPs, – MgCl <sub>2</sub> (5.5 mM final concentration), – Rox Passive reference – Stabilizers
Buffer S Blue cap	2 x 1.6 mL UFD-RPRT-C0101	RNase free glycerol buffer
	1 x 1.6 mL UFD-RPRT-C0100	RNase free glycerol buffer
PCR grade water Clear cap	1 x 2 mL	RNase free PCR grade water

Table 2: kit content

#### Resuspension

Resuspend the Takyon™ Dry MasterMix as per follow:

1) Add the appropriate volume of buffer S or PCR grade water (see table 1 for stability details) as described in the table 3

Final MasterMix concentration	Resuspension volume
2 X	525 µL (PCR grade water or buffer S)
5 X	210 µL (PCR grade water or buffer S)

Table 3: resuspension

2) Close the bottle and stir gently for 15 sec.

#### Procedure

1) Prepare the reaction mix (see table 4). Reaction set up should ideally be done on ice. To correct for dispensing losses, prepare an excess of reaction mix (e.g. a 50-reaction mix for 48 reactions).

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

Component	Volume (µL)	Final Concentration
Takyon™ RT MasterMix	10 (2X) 4 (5X)	1x
Forward primer	2	50-900 nM <sup>1</sup>
Reverse primer	2	50-900 nM <sup>1</sup>
Probe	2	100-250 nM <sup>1</sup>
Water	(volume is 20 µL minus all other components) <sup>2</sup>	
Total Mix / reaction	17.5 µL <sup>2</sup>	

Table 4: Mix preparation

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 optimisation of the primer concentrations and primer-probe ratio may be required to obtain the best results with your primer-probe set (see table 7).

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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 2, 3 and 4. These volumes, including primers & probes, can be adjusted depending on the template and reaction volumes.

- 3) Add the reaction mix to individual reaction vials.
- 4) Add the template to individual reaction vials, gently mix and centrifuge to avoid bubbles. Negative control containing no RNA template should always be included.
- 5) The Takyon™ Dry Rox One-Step RT Probe MasterMix will produce consistent and sensitive results under FAST and REGULAR cycling conditions. Set up the Real-Time thermocycler using the following recommended parameters (table 5):

		FAST cycling*	Regular Cycling
a) reverse transcription	48°C	10 min.	10 min.
For difficult templates, increase RT step by increment of 10', up to a total of 30', to improve reaction yield			
b) c-DNA amplification step			
Takyon™ activation	95 °C	3 min.	3 min.
<b>40 Cycles</b>			
Denaturation	95 °C	3 sec.**	10 sec.
Annealing / extension	60 °C ***	20 - 30 sec.	45 - 60 sec.

Table 5: Reaction setup

\* Only perform fast cycling on FAST cyclers equipped with a FAST block. Short amplicons (<120 bp) are recommended to support FAST cycling conditions. For longer amplicons or difficult templates, increase the annealing-extension time up to 40 sec. Example of FAST cyclers: LC480, RotorGenes, ABI 7500 & 7900 with FAST block (optional), ViiA7, ABI StepOne Plus, Quant-Studio...

\*\* For complex templates increase the denaturation time up to 15 sec per cycle during the first 10 cycles for optimal template denaturation. This parameter can be subject to optimisation.

\*\*\* The annealing temperature will vary depending on the melting temperature (Tm) of the primers.

Note that some FAST thermocyclers can accommodate shorter annealing steps for faster qPCR results. However some assays may require longer extension times for efficient amplification. Increase extension time by increments of 10 sec., if required.

## 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

Titrate according to the Table 6, 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.

Reverse	Forward		
	50 nM	300 nM	900 nM
50 nM	50 / 50	300 / 50	900 / 50
300 nM	50 / 300	300 / 300	900 / 300
900 nM	50 / 900	300 / 900	900 / 900

Table 6: Primer titration matrix

#### 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.

Probe			
Optimal primers concentration	50 nM	100 nM	250 nM

Table 7: Primer-probe ratio matrix

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