EUROPE ***Eurogentec*** LIEGE SCIENCE PARK • 4102 Seraing • BELGIUM
Tel.: +32 4 372 74 00 • Fax: +32 4 372 75 00 • Toll-free: + 800 666 00 123
info@eurogentec.com • www.eurogentec.com

NORTH AMERICA ***AnaSpec*** - 34801 Campus Drive • Fremont, CA 94555 • USA
Tel.: +1-510-791-9560 Toll-free: +1 800-452-5530 • Fax: +1 510-791-9572
service@anaspec.com • www.anaspec.com

Eurogentec
Experience true partnership

Product Data Sheet

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

Takyon™ Dry Low Rox Probe MasterMix dTTP

UFD-LPMT-C0101 ● UFD-LPMT-C0100

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

Storage condition

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

After resuspension the Takyon™ Dry Low Rox Probe MasterMix dTTP may be stored as per follow (Table1):

Format		*Temperature*	*Validity period*
Dried		15-35°C	18 months*
Liquid	in PCR grade water	4°C	24 hours
		-20°C	6 months
	in Buffer S	4°C	1 month
		-20°C	6 months

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 Low Rox Probe MasterMix dTTP 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-LPMT-C0101	1 bottle of MasterMix (50 RXN) contains *e.g.*: – Takyon™ DNA polymerase, – dNTPs, – MgCl2 (5.5 mM final concentration), – Rox Passive reference – Stabilizers
	1 x 50 RXN (20 µL) UFD-LPMT-C0100	
Buffer S Blue cap	2 x 1.6 mL UFD-LPMT-C0101	RNase free glycerol buffer
	1 x 1.6 mL UFD-LPMT-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 dTTP 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.

Reaction set up

1) Prepare the reaction mix (see table 4). 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™ MasterMix	10 (2X) 4 (5X)	1x
Forward primer	2	50-900 nM[1]
Reverse primer	2	50-900 nM[1]
Probe	2	100-250 nM[1]
Water	volume is 20 µL minus all other components[2]	
Total Mix / reaction	**17.5 µL**[2]	

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).

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

3) 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.

4) 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.

5) The Takyon™ Dry Low Rox Probe MasterMix dTTP 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
Takyon™ activation	95 °C	3 min.	3 min.
40 Cycles			
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 equiped 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 Prism® 7500 & 7900 with FAST block (optional), ViiA7, ABI StepOne® Plus, Quant-Studio,...

** For complex templates (*e.g.* plant DNA, large genomes…) it is recommended to increase the denaturation time up to 30 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 betwen 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 adjustement 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

For further information please contact our Customer Help Desk

EU : info@eurogentec.com +32 4 372 76 65 – **US :** service@anaspec.com +1 510 791 9560