EUROPE
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

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Technical **Data Sheet**

Takyon® One-Step Rox Probe 5X MasterMix dTTP

UF-RP5X-RT0501 · UF-RP5X-RT0505 · UF-RP5X-RT0510
[5 mL] [5 x 5 mL] [10 x 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!

Kit contents (Table 1)

The kit UF-RP5X-RT0501 (UF-RP5X-RT0101) contains enough reagents for up to 1250 (150) - 20 µL reactions using the performant hotstart Takyon® enzyme.

Table 1

Reagent	Volume	Description
5x MasterMix (blue cap)	5 x 1 mL	Five tubes of 5X reaction mix containing *e.g.*: – Takyon® DNA polymerase, – $MgCl_2$ (5.5 mM final concentration), – dNTPS, – Rox Passive reference, – Stabilizers.
50 mM $MgCl_2$ (clear cap)	1.5 ml	50 mM $MgCl_2$ solution (optional use)
Euroscript II RT/ RNAse inhibitor (white cap)	250 µL (30 µL for UF-RP5X-RT0101)	50 u/µL RT 20 u/µL RNAse inh.
Additive (blue cap)	250 µL (30 µL for UF-RP5X-RT0101)	Improve results on some viral and FFPE samples

Storage conditions

5X MasterMix component of the Takyon® One-Step Rox Probe 5X MasterMix dTTP should be stored between -15 °C and -25°C in a constant temperature freezer. When stored under these conditions, this component is stable for 2 years. For short term storage the Takyon® One-Step Rox Probe 5X MasterMix dTTP can be stored at 4 °C for 6 months.

- For long term storage, the Euroscript II RT & additive should be stored at a temperature between -65°C and -75°C in a constant temperature freezer. When stored under these conditions, the reagents are stable for 2 years. The Euroscript II RT and additive can be stored between -15°C and -25°C for up to 12 months.

Procedure

1- Thaw all required reagents completely and put them on ice except for the Euroscript II RT and RNase inhibitor, which should be kept in the freezer until required for use. Mix all reagents well by inversion and spin them down prior to pipetting.

2- Prepare the reaction mix (see Table 2). Reaction set up should be done on ice. 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 in the order in which they are presented in the table 2. Mix thoroughly by pipetting or inversion. Spin down.

Table 2

Component	Volume (µL)	Final Concentration
Takyon® MasterMix	4	1x
Forward primer	2	50-900 nM[1]
Reverse primer	2	50-900 nM[1]
Probe	2	100-250 nM[1]
Euroscript II RT	0.2 µL	10u
Additive (optional)[3]	0.2 µL	
RNAse-free Water	up to 17.5 µL	(volume is 20 µL minus all other components)[2]
Total Mix / reaction	**17.5 µL [2]**	

4- Add the reaction mix to individual reaction vials.

5- Add the template to individual reaction vials, gently mix on a magnetic stirrer and centrifuge to avoid bubbles. Negative control containing no RNA template should always be included. Optionally, a no RT-control should be set up in tubes / wells, which does not contain the EuroScript II RT/RNase Inhibitor.

6- The Takyon® One-Step Rox Probe 5X 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

Technical information

		FAST cycling* FAST ramping rates! - Only on FAST cyclers	Regular Cycling Regular ramping rates!
	T°C	**Time**	
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.**
40 Cycles			
Denaturation	**95 °C**	**3 sec.**	**10 sec.**
Annealing / extension	**60 °C ****	**20 - 30 sec.**	**45 - 60 sec.**

*** 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 7500 & 7900 with FAST block (optional), ViiA7, ABI StepOne Plus, MasterCycler ep realplex with FAST block (optional),...
** 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 5-second, if required.

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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 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

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

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

	Probe		
Opt. primers conc.	**50 nM**	**100 nM**	**250 nM**

$MgCl_2$ adjustment matrix

Standard $MgCl_2$ concentration is 5.5 mM but optimal $MgCl_2$ concentration can vary between assays. If necessary adjust the $MgCl_2$ concentration with the provided 50 mM $MgCl_2$ tube. Always prefer optimizing the primer and probe concentrations before the $MgCl_2$ concentration.
Adjust the amount of water if $MgCl_2$ is added to the reaction.

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

TDS-UF-RP5X-RT-1125-v5

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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 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 volume.