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Technical Data Sheet

One-Step Takyon® Ultra Probe 4X MasterMix

UF-UPRT-C0201 • UF-UPRT-C1001 • UF-UPRT-C1005 • UF-UPRT-C1010

[1 mL; 200 Rxns] [5 x 1 mL; 1 000 Rxns] [25 x 1 mL; 5 000 Rxns] [50 x 1 mL; 10 000 Rxns]

Kit contents (Table 1)

The kit contains enough reagents for **200** - 20 µL rxn per ml of MasterMix using the performant hotstart Takyon® enzyme.

Table 1

Reagent	Volume	Description
4x MasterMix (black cap)	1.05 mL for UF-UPRT-C0201	4x reaction mix contains – Takyon® DNA polymerase, – MgCl ₂ , – dNTPs, – Stabilizers.
	5 x 1.05 mL for UF-UPRT-C1001	
Euroscript II RT PLUS (white cap)	90 µl for UF-UPRT-C0201 2 x 220 µl for UF-UPRT-C1001	Blend of Euroscript II, RNAse inhibitor and stabilizers
ROX (red cap)	0.5 mL	For signal normalization

Procedure

IMPORTANT: convert your MasterMix to the appropriate ROX level before first use. The following table indicates the appropriate quantity of ROX passive reference to add per tube (1.05 ml) of One-Step Takyon® Ultra Probe 4X MasterMix depending on the thermocycler used. ROX passive reference is not required for thermocyclers not listed here-below.

qPCR Platform used	ROX quantity to be added
Mx3000P® Mx3005P® Mx4000®	16.8 µL
ABI Prism® 7500 ABI Prism® FAST 7500 ViiA7™ QuantStudio™	5.9 µL
ABI Prism® 5700 ABI Prism® 7000 ABI Prism® 7300 ABI Prism® 7700 ABI Prism® 7900 ABI Prism® FAST 7900 ABI Step One ABI Step One Plus MasterCycler® ep realplex I & II (rev. =< 2.1)	84 µL

1- Prepare or thaw all required reagents completely except for the Euroscript II RT PLUS, which should be kept in the freezer or on a cold block until required for use.

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

For increased convenience, a stable working blend of MasterMix and Euroscript II PLUS can be prepared. See last paragraph in section "Storage Conditions".

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	5	1x
Forward primer	2	50-900 nM ¹
Reverse primer	2	50-900 nM ¹
Probe	2	100-250 nM ¹
Euroscript II RT PLUS	0.4 µL	10u
RNAse-free Water	x µL	
Total Mix / reaction	Volume is 20 µl minus all other components 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 PLUS.

6- The One-Step Takyon® Ultra Probe 4X MasterMix will produce consistent and sensitive results under FAST cycling conditions. Program the Real-Time thermocycler using the following recommended parameters (Table 3):

Table 3

	T°C	Time*
a) Reverse transcription	50°C	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.
40 Cycles		
Denaturation	95°C	15 sec.***
Annealing/extension	60°C**	30 sec.***

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* **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, QuantStudio™, CFX96,...

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

*** 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. Likewise, simple templates can accommodate shorter denaturation steps (5"), whilst complex templates like plant nucleic acids may require longer (up to 30") denaturation steps during initial cycles.

Technical information

Primer and probe design guidelines

Probes:

- Avoid runs of identical nucleotides, especially of 4 or more Gs.
- The probe T_m should be 7 to 10 °C above primers T_m.
- 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 T_m.

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 T_m 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 250 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 4, perform qPCR and select the concentration which gives the lowest C_q 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 C_q value.

Table 5: Primer-probe ratio matrix

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

Storage conditions

The One-Step Takyon® Ultra Probe 4X MasterMix should be stored between -20°C and -25°C in a constant temperature freezer. When stored under these conditions, the kit is stable for 24 months.

For short term storage the MasterMix component of the One-Step Takyon® Ultra Probe 4X MasterMix can be stored at 4°C for 6 months.

The Euroscript II RT PLUS should always be stored at a temperature between -20°C and -25°C in a constant temperature freezer.

For increased convenience, a working blend of MasterMix and Euroscript II RT Plus (a "one-tube solution") can be prepared and stored at -20°C for up to one month. Moreover, this blend will be stable at 20°C for up to 24h allowing for time-consuming high-throughput assays set-up. Combine 84 µl of Euroscript II RT Plus with 1.05 ml MasterMix. Use 5.4 µl of this blend per 20 µl final reaction as per Table 2.

For further information please contact our Customer Help Desk:

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Note 1: Primers 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: The template is added to the reaction mix prior 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.