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Technical **Data Sheet**

Takyon® Ultra Probe 4X qPCR MasterMix

UF-UPMT-C0200 • UF-UPMT-C0201 • UF-UPMT-C1001 • UF-UPMT-C1005 • UF-UPMT-C1010

[1 mL; 200 Rxns] [5 x 1 mL; 1 000 Rxns] [5x(5 x 1 mL); 5 000 Rxns] [10x(5 x 1 mL); 10 000 Rxns]

Kit contents (Table 1)

Each MasterMix tube contains enough reagents for **200** - 20 µL reactions using the performant hotstart Takyon® enzyme.

Table 1

Reagent	Volume	Description
4x MasterMix (black cap)	1.05 mL for UF-UPMT-C0201 5 x 1.05 mL for UF-UPMT-C1001	4x reaction mix contains – Takyon® DNA polymerase, – $MgCl_2$, – dNTPs, – Stabilizers.
ROX (red cap)	0.5 mL	For signal normalization

UF-UPMT-C1005 = 5 packs of UF-UPMT-C1001 ; UF-UPMT-C1010 = 10 packs of UF-UPMT-C1001

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 Takyon® Ultra Probe 4X qPCR MasterMix depending on the thermocycler used. ROX passive reference is not required for thermocyclers not listed here-below.

qPCR Plateform 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.

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).

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]
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 DNA template should always be included.

6- The Takyon® Ultra Probe 4X qPCR 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*
Takyon® activation	95°C	3 min.
40 Cycles		
Denaturation	95°C	15 sec.***
Annealing/extension	60°C**	30 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, QuantStudio™, CFX96,...
** The annealing temperature will vary depending on the melting temperature (Tm) 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 accomodate shorter denaturation steps (5"), whilst complex templates like plant nucleic acids may require longer (up to 30") denaturation steps during intial cycles.

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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 250 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**

Storage conditions

The Takyon® Ultra Probe 4X qPCR 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 4X qPCR MasterMix can be stored at 4°C for 6 months.

For further information please contact our Customer Help Desk:

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For technical questions, please contact scientific: support@eurogentec.com

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.