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Technical Data Sheet

Takyon® Low Rox Probe MasterMix UNG

UF-LPMU-C0101 [1.5 ML] • UF-LPMU-C0701 [7.5 ML]
• UF-LPMU-C0705 [5X 7.5 ML] • UF-LPMU-C0710 [10X 7.5 ML]

Emerging from the combination of an optimized reaction buffer and the efficient « Takyon® » enzyme, Takyon® kits for Probe Assays ensure sensitivity and fast delivery of accurate and reproducible results.

Storage conditions

For long term storage the Takyon® Low Rox Probe MasterMix UNG should be stored at a temperature between -15 °C and -25 °C in a constant temperature freezer. When stored under these conditions, the components are stable for 24 months. For short term storage the Takyon® Low Rox Probe MasterMix UNG can be stored at 4 °C for 6 months.

Kit contents (Table 1)

The kit UF-LPMU-C0701 (UF-LPMU-C0101) contains enough reagents for up to 750 (150) - 20 µL reactions using the hotstart Takyon® enzyme.

TABLE 1

Component	Volume	Description
2x MasterMix (yellow cap)	7.5 mL 1.5 mL for UF-LPMU-C0101	– One tube/bottle of 2x reaction mix containing e.g.: – Takyon® DNA polymerase, – $MgCl_2$ (5.5 mM final concentration), – A blend of dATP, dCTP, dGTP and dTTP/dUTP – Rox Passive reference (low concentration) – Uracil-N-glycosylase – Stabilizers
50 mM $MgCl_2$ (clear cap)	1.5 mL	50 mM $MgCl_2$ solution (optional use)

Procedure

1. Thaw all required reagents completely and put them on ice. Mix all reagents well by inversion and spin them down prior to pipetting.

2. Prepare the reaction mix (see Table 2). 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. Mix thoroughly by pipetting or inversion. Spin down.

TABLE 2

Component	Volume (µL)	Final Concentration
Takyon® MasterMix	10	1x
Forward primer	2	50-900 nM[1]
Reverse primer	2	50-900 nM[1]
Probe	2	100-250 nM[1]
Water	1.5	(volume is 20 µL minus all other components)[2]
Total Mix / reaction	17.5 µl[2]	

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/control DNA 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 volumes.

4. 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.

5. 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.

6. The Takyon® Low Rox Probe MasterMix UNG 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

		FAST cycling* FAST ramping rates – Only on FAST cyclers	Regular Cycling - Regular ramping rates
	T°C**	Time	
Carry over prevention	50 °C	2 min.	2 min.
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 equipped with a FAST block. Short amplicons (<120 bp) are recommended to support FAST cycling conditions. For longer amplicons or difficult templates,

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increase the annealing-extension time up to 40 sec. Examples of FAST cyclers: LC480, RotorGenes, ABI 7500 & 7900 with FAST block (optional), ViiA7, ABI StepOne Plus.

** It is essential to avoid using UNG in PCR cycles where the annealing/extension temperatures are below 55°C to ensure optimal and consistent results. Temperatures of at least 55°C should be used throughout the cycling protocol to avoid degrading the PCR products.

*** 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.

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 center 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 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

	Forward		
Reverse	**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

	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.

Further information available through Eurogentec website: www.eurogentec.com.

For further information please contact our Customer Help Desk:

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