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Technical **Data Sheet**

Takyon® Rox Probe Core Kit dTTP

UF-RPCT-C0201 · UF-RPCT-C0205 · UF-RPCT-C0210

[1250 RXN - 20µL] [5 x 1250 RXN - 20µL]] [10 x 1250 RXN - 20µL]]

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!

Storage conditions

For long term storage the Takyon® Rox Probe Core Kit dTTP 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® Rox Probe Core Kit dTTP can be stored at 4 °C for 6 months.

Kit contents (Table 1)

The kit UF-RPCT-C0201 contains enough reagents to prepare up to 1250 - 20 µL reactions using the performant hotstart Takyon™ enzyme.

Table 1

Reagent	Volume	Description
10x Buffer tube (red cap)	2 x 1.5 mL	One tube of 10x reaction buffer contains: – KCl and Tris-HCl – Stabilizers, – Rox Passive reference
50 mM $MgCl_2$ (clear cap)	2 x 1.5 mL	50 mM $MgCl_2$ solution (optional use)
5 mM dNTPs (green cap)	1 x 1.25 mL	A blend of dATP, dCTP, dGTP and dTTP (200µM each)
Takyon® enzyme (yellow cap)	1 x 125 µL	Takyon® enzyme (5 U/µL)
dUTP/UNG mix	1 x 330 µL	dUTP and Uracyl N-glycosylase blend for carryover prevention

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
10x reaction buffer	2	1x
Forward primer	2	50-900 nM[1]
Reverse primer	2	50-900 nM[1]
Probe	2	100-250 nM[1]
50 mM $MgCl_2$	2.2	5.5 mM
5mM dNTP mix	0.8	200 µM of each dNTP
Takyon® 5U/µL	0.1	0.02 U/µL
dUTP/UNG additive[2]	0.25	Optional
Water	6.15	Water volume is 17,5 µL minus volume of all other components
Total Mix / reaction	17.5 [3]	
Template or Control	2.5[3]	

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® Rox Probe Core Kit 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

		FAST cycling* Only on FAST cyclers	**Regular Cycling**
	T°C	**Time**	
Carry over prevention optional**	**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 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),...
** dUTP/UNG blend must be added to the reaction mix (see table 2).
*** 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.

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: If carry over contaminations is a concern optionally add 0.25µL of the dUTP/UNG additive per 20µL of reaction. In the event of dUTP/UNG addition, 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.

Note 3: 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 and probes, can be adjusted depending on the template and reaction volumes.

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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 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:

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