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Eurogentec

Technical **Data Sheet**

Takyon® No Rox SYBR® Core Kit dTTP Blue

UF-NSCT-B0201 • UF-NSCT-B0205 • UF-NSCT-B0210
[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 SYBR® Assays ensure sensitivity and fast delivery of accurate and reproducible results!

Storage conditions

For long term storage the Takyon® No Rox SYBR® Core Kit dTTP blue 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® No Rox SYBR® Core Kit dTTP blue can be stored at 4°C for 6 months. The SYBR® Green I is light sensitive and should be kept away from light as much as possible.

Kit contents (Table 1)

The kit UF-NSCT-B0201 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 (brown cap)	2 x 1.5 mL	One tube of 10x reaction buffer contains: – KCl and Tris-HCl – Stabilizers, – Inert Blue dye
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
Takyon® enzyme (yellow cap)	1 x 125 µL	Takyon® enzyme (5 U/µL)
DMSO	1 mL	One tube of DMSO
dUTP/UNG mix	1 x 330 µL	dUTP and Uracyl N-glycosylase blend for carryover prevention
SYBR® Green I (amber tube)	-	One tube of SYBR® Green I stock

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. Prepare a dilution of SYBR® Green I (store at 4°C in the dark). Briefly microcentrifuge the SYBR® Green I Stock. Add the DMSO completely. Mix to give a working solution

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).

Table 2

Component	Volume (µL)	Final Concentration
10x reaction buffer	2	1x
Forward primer	2	50-300 nM[1]
Reverse primer	2	50-300 nM[1]
Diluted SYBR®	0.6	-
50 mM $MgCl_2$	1	2.5 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	8.75	Water volume is 17,5 µL minus volume of all other components
Total Mix / reaction	17.5 [3]	
Template or Control	2.5[3]	Total volume of 20 µL

3- Add all components together, except for the template. Mix thoroughly by pipetting or inversion. Spin down.

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® No Rox SYBR® Core Kit dTTP blue 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.**

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), CFX 96/384...
** 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 concentration of 100 nM is recommended as starting concentration. This concentration will be correct for many assays, but additional optimization of the primer concentration may be required to obtain the best results with your prime set (see table 5).

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, can be adjusted depending on the template and reaction volumes.

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Table 4:
3-Step cycling protocol for maximal sensitivity

		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	**60 °C** ***	**15 sec.**	**20 sec.**
Extension	**72 °C**	**15 sec.**	**20-40 sec.**

Technical information

Primer and probe design guidelines

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 concentration for primers is 100 nM. Optimal results may require titration of primers or adjustement of the primer ratio. The purpose of such a process is to determine the minimum amount of primers required to obtain the most sensitive results with your assay.

Primer titration matrix

Titrate according to the Table 5, 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 5:
Primer titration matrix

Reverse	**Forward**		
	50 nM	**100 nM**	**300 nM**
50 nM	50 / 50	100 / 50	300 / 50
100 nM	50 / 100	100 / 100	300 / 100
300 nM	50 / 300	100 / 300	300 / 300

$MgCl_2$ adjustment matrix

Standard $MgCl_2$ concentration is 2.5 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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