EUROPE
LIEGE SCIENCE PARK • 4102 Seraing • BELGIUM • Tel.: +32 4 372 74 00 • Fax: +32 4 372 75 00
Toll-free: + 800 666 00 123 • info@eurogentec.com • www.eurogentec.com

NORTH AMERICA
ANASPEC - 34801 Campus Drive • Fremont, CA 94555 • USA • Tel.: +1-510-791-9560
Toll-free: +1 800-452-5530 • Fax: +1 510-791-9572 • service@anaspec.com
www.anaspec.com

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Technical **Data Sheet**

Takyon™ Low Rox SYBR® MasterMix dTTP Blue

UF-LSMT-B0101 • UF-LSMT-B0701 • UF-LSMT-B0705 • UF-LSMT-B0710
[1.5 mL] [7.5 mL] [5 x 7.5 mL] [10 x 7.5 mL]

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™ Low Rox SYBR® MasterMix 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 12 months. For short term storage the Takyon™ Low Rox SYBR® MasterMix dTTP blue can be stored at 4 °C for 6 months. Product must be stored in the dark.

Kit contents (Table 1)

The kit UF-LSMT-B0701 (UF-LSMT-B0101) contains enough reagents for up to 750 (150) - 20 µL reactions using the performant Hotstart Takyon™ enzyme.

Table 1

Reagent	Volume	Description
2x MasterMix (red cap - amber tube or vial)	7.5 mL 1.5 mL for UF-LSMT-B0101	One tube/bottle of 2x reaction buffer contains: – Takyon™ DNA polymerase, – $MgCl_2$ (2.5 mM final concentration), – SYBR Green®, – dNTPs, – Rox Passive reference (low concentration) – Inert blue dye, – 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	50-300 nM[1]
Reverse primer	2	50-300 nM[1]
Water	3.5	(volume is 20 µL minus all other components)[2]
Total Mix / reaction	**17.5 µL** [2]	

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 SYBR® MasterMix 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),CFx96/384...
** For carryover prevention, add 200µL dUTP/UNG blend (RT-UTP UNG-020) in 7.5 mL MasterMix (optional).
*** 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: Primers concentration of 100 nM is recommended as a starting concentration. This concentration will be correct for many assays, but additional optimization of the primers concentration may be required to obtain the best results with your primer set (see table 5).

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

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Table 4:
3-Step cycling protocol for maximal sensitivity

	T°C	Time	
		Fast	Regular
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 design guidelines

- 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.

Custom assay design

The commonly used concentrations for primers are 100 nM. Optimal results may require titration of primers or adjustment of the ratio. The purpose of such a process is to determine the minimum amount of primers required obtaining 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 and clear NTC's. 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:

For Europe:
E-mail: info@eurogentec.com
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For USA:
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Tel.: +1-510-791-9560 • Toll-free: +1-800-452-5530

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