

Takyon® Low Rox SYBR® MasterMix dTTP Blue¹

UF-LSMT-B0101

[1.5 mL]

UF-LSMT-B0701

[7.5 mL]

UF-LSMT-B0705

[5 x 7.5 mL]

UF-LSMT-B0710

[10 x 7.5 mL]

Kit content (for 750 (150) - 20 µL reactions)

INCLUDED



2× Takyon® MasterMix

 ● Red cap + ● Amber tube/vial
[7.5 mL]

 [1.5 mL for **UF-LSMT-B0101**]

OPTIONAL



1× 50 mM MgCl₂

● Clear cap

[1.5 mL]

NOT INCLUDED


Forward primer

Reverse primer

Water

- Takyon® DNA polymerase
- MgCl₂ (2.5 mM final concentration)
- SYBR® Green
- dNTPs
- Rox Passive reference (low concentration)
- Inert blue dye
- Stabilizers

Storage

SHORT TERM STORAGE

6-month stability

In the dark after kit production date

4°C

LONG TERM STORAGE

12-month stability

In the dark after kit production date

-15°C | - 25°C

qPCR reagents containing SYBR® Green should be protected from light during storage and qPCR assay setup.

Optimization tips

Refer to the primer design guidelines, custom assay design recommendations, primer titration matrix, and MgCl₂ adjustment protocols for best results.

Upon developing a new assay or changing qPCR reagent kit, conducting a primer matrix may be required to ensure optimal performance.

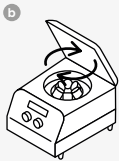
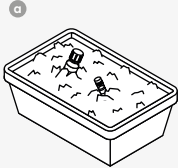


**TECHNICAL
INFORMATION**

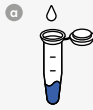
[1] Eurogentec products are sold for research or laboratory use only and are not to be administered to humans or used for medical diagnostics.

Recommended protocol for 20- μ L reactions

1 **Thaw all required reagents** completely and put them **on ice**. Mix all reagents well by inversion and **spin down** the tube(s) prior to pipetting.



2 **Prepare the REACTION MIX** in excess to correct for dispensing losses (e.g. a 100-reactions mix for 96-reactions). **Add all components together**, except for the template and controls. Mix gently by pipetting or inversion. **Spin down**.



(a)



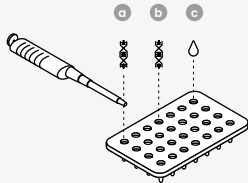
(b)



(c)

REACTION MIX COMPONENTS	2x Takyon® MasterMix	Forward primer	Reverse primer	Water	Total Volume
VOLUME / REACTION	10 μ L	2 μ L	2 μ L	3.5 μ L ²	17.5 μ L ⁴
FINAL CONCENTRATION	1x	50-300 nM ³	50-300 nM ³	(volume is 20 μ L minus all other components) ²	

3 **Pipette** into your qPCR plate/ vials either:

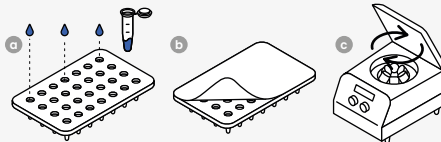


(a) 2.5 μ L of template CDNA / DNA

(b) 2.5 μ L of positive control

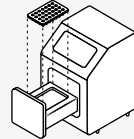
(c) 2.5 μ L of water / buffer for negative control

4 **Add 17.5 μ L 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.

5 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:



	T ^o C	Time	FAST CYCLING ⁵	REGULAR CYCLING
Carry over prevention ⁶ (Optional)	50 ^o C	2 min		2 min
Takyon® activation	95 ^o C	3 min		3 min
40 Cycles⁷				
2- STEPS	Denaturation ⁸	95 ^o C	3 sec	10 sec
	Annealing / extension ⁹	60 ^o C	20 - 30 sec	45 - 60 sec
3- STEPS	Denaturation ⁸	95 ^o C	3 sec	10 sec
	Annealing ⁹	60 ^o C	15 sec	20 sec
	Extension	72 ^o C	15 sec	20 - 40 sec

[2] Water volume is 20 μ L minus volume of all other components. [3] 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. [4] 17.5 μ L of reaction mix is added to 2.5 μ L of template/control DNA prior to cycling, giving a final reaction volume of 20 μ L. See steps 3 and 4. These volumes, including primers and probes, can be adjusted depending on the template and reaction volumes. [5] 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, increase the annealing-extension time up to 40 sec. [6] Applicable only if the dUTP/UNG Additive (Ref. RT-UTPUNG) has been added to the Master-Mix. In such case, a temperature of at least 55^oC should be maintained throughout the cycling protocol. [7] A 2-step protocol is recommended and effective in most cases. For challenging assays, optimize the primer matrix before considering a 3-step protocol. [8] Complex templates (plant DNA, genomic DNA...) may require a longer denaturation time. [9] The annealing temperature will vary depending on the melting temperature (T_m) 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.