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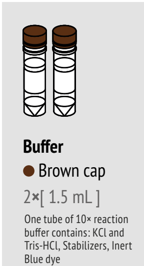
Takyon® No Rox SYBR® Core Kit dTTP Blue[1]

UF-NSCT-B0201	UF-NSCT-B0205	UF-NSCT-B0210
[1250 RXN - 20μL]	[5 x 1250 RXN - 20μL]	[10 x 1250 RXN - 20μL]

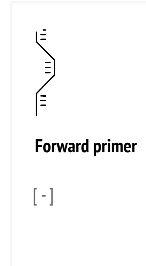
Kit content (for 1250 - 20 μL reactions)

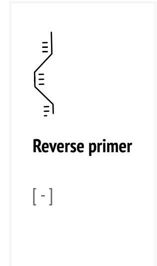
INCLUDED

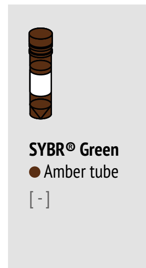


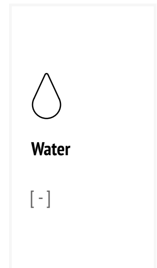
OPTIONAL

NOT INCLUDED









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 $MgCl_2$ 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 administrated to humans or used for medical diagnostics.

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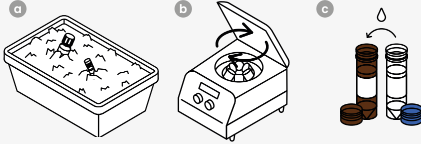
Recommended protocol for 20-μL reactions

1 (a) **Thaw all** required **reagents** completely and put them **on ice**. Mix all reagents well by inversion and (b) **spin** them **down** prior to pipetting. (c) **Briefly microcentrifuge the SYBR® Green I stock**, then **dilute it by adding DMSO completely** (store at 4°C in the dark).

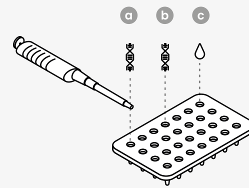


2 (a) **Prepare the REACTION MIX** in excess to correct for dispensing losses (*e.g.* a 100-reactions mix for 96-reactions).
Add all components together, except for the template. Mix thoroughly by pipetting or inversion. **Spin down.**

(a)

REACTION MIX COMPONENTS	VOLUME / REACTION	FINAL CONCENTRATION
Buffer	2 μL	1×
Forward primer	2 μL	50-300 nM[3]
Reverse primer	2 μL	50-300 nM[3]
Diluted SYBR®	0.6 μL	-
50mM $MgCl_2$	1 μL	2.5 mM
5mM dNTP mix	0.8 μL	200 μM of each dNTP
Takyon® 5U/μL	0.1 μL	0.02 U/μL
dUTP/UNG additive[2]	0.25 μL	Optional
Water	8.75 μL[2]	(volume is 17,5 μL minus all other components)[2]
Total Volume	**17.5 μL[4]**	
Template or Control	2.5 μL[4]	Total volume of 20 μL

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

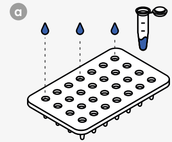


(a) **2.5 μL of template CDNA /DNA**

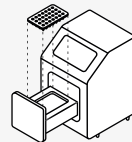
(b) **2.5 μL of positive control**

(c) **2.5 μL of water / buffer for negative control**

4 (a) **Add 17.5 μL** of the **REACTION MIX** per well or 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® 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:



		T°C	FAST CYCLING[5] Time	REGULAR CYCLING
	Carry over prevention[6] (Optional)	50°C	2 min	2 min
	Takyon® activation	95°C	3 min	3 min
	40 Cycles[7]			
2 STEPS	Denaturation[8]	95°C	3 sec	10 sec
2 STEPS	Annealing / extension[9]	60°C	20 - 30 sec	45 - 60 sec
3 STEPS	Denaturation[8]	95°C	3 sec	10 sec
3 STEPS	Annealing[9]	60 °C	15 sec	20 sec
3 STEPS	Extension	72 °C	15 sec	20 - 40 sec

[2] Water volume is 20 μL 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 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 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]** dUTP/UNG blend must be added to the reaction mix only when a temperature of at least 55°C is 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 (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 seconds, if required.

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