

# qPCR Core kit for SYBR® Green I No ROX

## Technical Data Sheet

**Reference: RT-SN10-05NR**

Products and procedures describe in this protocol are intended for research purposes only.

### Storage conditions

For long term storage the qPCR Core kit for SYBR® Green I No ROX should be stored at -15°C to -25°C in a constant temperature freezer. When stored under these conditions the reagents are stable for 1 year.

For short term storage the qPCR Core kit for SYBR® Green I No ROX 10x reaction buffer, MgCl<sub>2</sub> and the prediluted SYBR® Green I can be stored at 4 °C to 6 °C for 1 month. The Taq polymerase should be stored at -15 °C to -25 °C.

The SYBR® Green I tube of a qPCR Core kit for SYBR® Green I No ROX should be protected from light whenever possible.

### Kit contents

The qPCR Core kit for SYBR® Green I No ROX contains enough reagents for up to 500 - 50 µl reactions using the hotstart enzyme, HotGoldStar.

Reagent	Volume	Description
10x Reaction buffer (brown cap)	2.8 ml	Two tubes (1.4 ml) of reaction buffer containing KCl, and Tris-HCl
50 mM MgCl <sub>2</sub> (plain cap)	1.5 ml	Two tubes of 50 mM MgCl <sub>2</sub>
5 mM dNTP Mix (green cap)	1.25 ml	One tube of dATP, dCTP, dGTP, dTTP and dUTP in autoclaved, deionised water, titrated with NaOH to pH 7.0
PCR enzyme (yellow cap)	125 µl	One tube containing 5 U/µl HotGoldStar
SYBR® Green I stock (amber tube)*	-	One tube of SYBR® Green I stock
DMSO (blue cap)	1 ml	One tube of DMSO

\*The SYBR® Green I is light sensitive and should be kept away from light as much as possible.

### Procedure

- 1- Thaw all required reagents completely and put them on ice. Mix all reagents well by inversion and spin them down prior to pipeting.
- 2- **Preparation of diluted 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

### 3- Prepare the reaction mix

Component	Volume (µl)	Final Concentration
10x reaction buffer	5	1x
50 mM MgCl <sub>2</sub>	3.5	3.5 mM or as required
5 mM dNTP mix	2	200 µM each dNTP
Forward primer	5 (initially)	(initially 100-300 nM)*
Reverse primer	5 (initially)	(initially 100-300 nM)*
HotGoldStar	0.25	0.025 U/µl
Diluted SYBR®	1.5	-
Template	5	-
Water	22.75	(Volume is 50 µl minus all other components)
<b>Total Mix</b>	<b>50 µl</b>	

\*Note1: the primer concentrations are recommended as starting concentrations; always start at the lower end. These concentrations will be correct for many assays, but additional optimization may be required to obtain the best results with your primer set.

Note 2: Uracil-N-Glycosylase (reference RT-0610-03) can be added to a final concentration of 0.01 U/µl if required (0.5 µl of 1 U/µl UNG per 50 µl reaction). If the UNG is required please add the following step at point 7 before the HotGoldStar activation step: 2 min at 50 °C.

- 4- To correct for dispensing losses prepare an excess of reaction mix (for example 100 reactions reaction mix for 96 reactions). Add all components together, except for the template. Mix thoroughly by inversion. Spin down.
- 5- Pipette 5 µl of the template DNA for your samples, 5 µl of the control DNA for your positive control and 5 µl of water or buffer for your negative control in to your PCR tubes / 96-well plate / 384-well plate.
- 6- Add 45 µl of the reaction mix to the reaction vial, close the vial and mix gently on a stirrer or spin down. Ensure that no bubbles are present in the reaction vial. Reaction set up can be done at room temperature.
- 7- Program the Real-Time thermocycler using the following recommended parameters:

<b>HotGoldStar activation</b>	10 min. 95 °C
<b>40 Cycles</b>	15 sec. 95 °C 1 min. 60 °C
<b>Hold</b>	50 °C forever

## 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
- using the Primer Express® software the T<sub>m</sub> should be 58 °C to 60 °C
- the primer should be placed as close as possible to the probe

### Custom assay design

Commonly used concentrations are 100 nM for primers. Optimal results may require titration of primers. 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 1, perform qPCR and select the concentration, which gives the lowest Ct 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 1:** 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<sub>2</sub> adjustment matrix

Standard MgCl<sub>2</sub> concentration is 3.5 mM but optimal MgCl<sub>2</sub> concentration can vary between assay. Always prefer optimizing the primer concentrations before the MgCl<sub>2</sub> concentration. Adjust the amount of water if MgCl<sub>2</sub> is added to the reaction.

Final MgCl <sub>2</sub> concentration (mM)	MgCl <sub>2</sub> to add (µl/50 µl)	10x reaction buffer (µl)
2.5	2.5	5
3	3	5
3.5	3.5	5
4	4	5
4.5	4.5	5
5	5	5
5.5	5.5	5
6	6	5

### 3-step protocol instead of 2-step protocol

Increasing extension time or performing a 3-step protocol can increase the ΔR<sub>n</sub> and / or decrease the Ct of an assay, particularly when the PCR product is longer than 100 bp.

The protocol will be as follows:

<b>HotGoldStar activation</b>	10 min. 95 °C
<b>40 Cycles</b>	denaturation 15 sec. 95 °C
	annealing 20 sec. 60 °C
	extension 40 sec. 72 °C
Increase extension time with 10-second steps, if required.	

Further information available through Eurogentec web site, [www.eurogentec.com](http://www.eurogentec.com).

- Manual for qPCR Core kit for SYBR® Green I No ROX, reference RT-0000-06 (under the "Technical Resources / Manual" section).
- Troubleshooting Guide for qPCR and RTqPCR (under the "Technical Resources / Troubleshooting Guide" section).
- Primers and probe design (please refer to our Troubleshooting Guide).
- "Your One-stop-shop Real-Time qPCR supplier" handbook (under the "Technical Resources / Documentation" section).
- MSDSs, (under the "Technical Resources / MSDS" section)
- Certificates of Analysis (please contact us).

**For any further information required please contact our Customer Help Desk:**

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