

# qPCR MasterMix for SYBR® Green I Technical Data Sheet

**Reference: RT-SN2X-03T**

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

## Storage conditions

For long term storage the qPCR MasterMix for SYBR® Green I 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 MasterMix for SYBR® Green I and the prediluted SYBR® Green I can be stored at 4 °C to 6 °C for 1 month. The SYBR® Green I diluted in DMSO can become solid at 4 °C.

The SYBR® Green I tube of a qPCR MasterMix for SYBR® Green I should be protected from light whenever possible.

## Kit contents

The qPCR MasterMix for SYBR® Green I contains enough reagents for up to 300 - 50 µl reactions using the hotstart enzyme, HotGoldStar.

Reagent	Volume	Description
2x reaction buffer (yellow cap)	7.5 ml	Five tubes (1.5 ml) of reaction buffer, dNTPs (including dUTP) HotGoldStar DNA polymerase, MgCl <sub>2</sub> (5mM final concentration), Uracil-N-Glycosylase, stabilizers and passive reference
50 mM MgCl <sub>2</sub> (plain cap)	1.5 ml	One tube of 50 mM MgCl <sub>2</sub>
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
2x reaction buffer	25	1x
Forward primer	5	(initially 100 up to 300 nM)*
Reverse primer	5	(initially 100 up to 300 nM)*
Diluted SYBR®	1.5	-
Template	5	-
Water	8.5	(volume is 50 µl minus all other components)
<b>Total Mix</b>	<b>50 µl</b>	

\*Note that 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.

- 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>UNG step</b>	2 min. 50 °C
<b>HotGoldStar activation / UNG inactivation</b>	10 min. 95 °C
<b>40 Cycles</b>	15 sec. 95 °C 1 min. 60 °C
Hold at 50 °C forever or perform a meltcurve	

## 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

### 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 5 mM but optimal MgCl<sub>2</sub> concentration can vary between assay, if necessary use the 50 mM MgCl<sub>2</sub> tube. 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)	2x reaction buffer (µl)
5	0	25
5.5	0.5	25
6	1	25

#### 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>UNG step</b>		2 min. 50 °C
<b>HotGoldStar activation / UNG inactivation</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 MasterMix for SYBR® Green I, reference RT-0000-02 (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:**

*For Europe:*

E-mail: [info@eurogentec.com](mailto:info@eurogentec.com)

Tel : +32 4 372 76 65

*For USA:*

E-mail: [info.usa@eurogentec.com](mailto:info.usa@eurogentec.com)

Tel: +1 858 793 26 66

#### FOR RESEARCH USE ONLY

##### NOTICE TO PURCHASER: LIMITED LICENSE

A license under U.S. Patents 4,683,202, 4,683,195 and 4,965,188 or their foreign counterparts, owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd (Roche), has an up-front fee component and a running-royalty component. The purchase price of this product includes limited, non-transferrable rights under the running-royalty component to use only this amount of the product to practice the Polymerase Chain Reaction ("PCR") and related processes described in said patents solely for the research and development activities of the purchaser when this product is used in conjunction with a thermal cycler whose use is covered by the up-front fee component. Rights to the up-front fee component must be obtained by the end user in order to have a complete license. These rights under the up-front fee component may be purchased from Applied Biosystems or obtained by purchasing an Authorized Thermal Cycler. No right to perform or offer commercial services of any kind using PCR, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is hereby granted by implication or estoppel. Further information on purchasing licenses to practice the PCR Process may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or the Licensing Department at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

Cyclic-substituted unsymmetrical cyanine dyes are covered by U.S. Patents 5,436,134 and 5,658,751 and licensed to Eurogentec S.A by Molecular Probes, Inc in the direct research field.

Use of UDG employs U.S. patents 5,035,996, 5,946,313, 5,683,896 and their foreign counterparts licensed to Eurogentec, S.A. from Invitrogen Corporation.

Primer Express® is a registered trademark of Applied Corporation.