

Eurogentec SA  
Parc scientifique du Sart Tilman  
4102 SERAING  
BELGIUM  
*Tel. +32 4 240 76 76 Fax. +32 4 254 07 88*



**E U R O G E N T E C**  
**EGT GROUP**

## **Lithos qPCR™ HotStart Mastermix Optimisation Kit**

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

**Store at -20°C in a constant temperature freezer.**

**Cat. No. RT-SNHS-OKLC**

LightCycler™ is a trademark of Idaho Technologies Inc.  
Sybr™ is a registered trademark of Molecular Probes Inc.

## Storage and Stability

Store the Lithos qPCR™ HotStart Optimisation Kit at -15 to -25°C in a constant temperature freezer.

## Kit Contents

The Lithos qPCR™ HotStart Optimisation Kit contains enough PCR reagents for up to 200 - 20µl reactions.

Reagent	Volume	Description
2X Master Mix (our coded as below)	2.0ml	four tubes of Tris-HCl, BSA, (Col-dNTP (inc. dUTP), Hot GoldStar DNA polymerase and MgCl <sub>2</sub> , 0.5ml each
MgCl <sub>2</sub> (Clear cap)	1.5ml	one tube of 25mM MgCl <sub>2</sub>
Sybr™ Green I (Amber Cap)	5µl	one tube of a 1,000 fold (1/10) stock solution of Sybr™ Green I
DMSO (Blue Cap)	0.25ml	one tube of DMSO

Red Cap	2mM final $\text{MgCl}_2$ - Cat. No. RT-SNHS-35LC2
Green Cap	3mM final $\text{MgCl}_2$ - Cat. No. RT-SNHS-35LC3
Yellow Cap	4mM final $\text{MgCl}_2$ - Cat. No. RT-SNHS-35LC4
Orange Cap	5mM final $\text{MgCl}_2$ - Cat. No. RT-SNHS-35LC5

## **Materials Required But Not Supplied**

The following items will be required in addition to the reagents supplied in the Lithos qPCR™ HotStart Optimisation Kit

### **Reagents**

Deionised or distilled water

Unlabelled primers

Labelled probe (or Sybr™ Green I if not supplied)

### **Materials**

LightCycler™ capillaries and caps

Microcentrifuge

Polypropylene tubes

Pipettes, preferably positive displacement

Pipette tips, with filter plugs

Vortexer

Disposable Gloves

## PCR Reaction Mix for labelled probes

Component	Volume ( $\mu$ l)	Final Concentration in 20 $\mu$ l)
2x Reaction Buffer	10	1x
Forward Primer	1	as required
Reverse Primer	1	as required
Labelled Probe	1	as required
Water	6	-
Template	1	-
<b>Total Mix</b>	<b>20<math>\mu</math>L</b>	

Note - Uracil-N-glycosylase (Cat. No. ME-0610-03) can be added to a final concentration of 0.01u/ul if required - 0.2 $\mu$ l of 1u/ $\mu$ l UNG per 20 $\mu$ l reaction.

Typical primer and probe concentrations are 0.5 $\mu$ M.

## Preparation of a 1 in 500 dilution of Sybr™ Green I

Briefly microcentrifuge the 5 $\mu$ l 1/10 dilution Sybr™ Green I stock (Amber Tube), add 0.245ml of DMSO (Blue capped Tube) and mix to give a 1 in 500 working stock.

## PCR Reaction Mix for Sybr™ Green I

Component	Volume (µl)	Final Concentration in 20µl)
2x Reaction Buffer	10	1x
Forward Primer	1	as required
Reverse Primer	1	as required
1in 500 diln. of Sybr™ Green I	0.5	1 in 20,000
Water	6.5	-
Template	1	-
<b>Total Mix</b>	<b>20µL</b>	

Note - Uracil-N-glycosylase (Cat. No. ME-0610-03) can be added to a final concentration of 0.01u/ul if required - 0.2µl of 1u/µl UNG per 20µl reaction.

Typical primer concentrations are 0.5µM.

## PCR Temperature Cycling Conditions

The Lithos qPCR™ HotStart Optimisation Kit has been optimised for performance on a Roche Diagnostics LightCycler™.

Typical profile times for this kit on this system using labelled probes are:

<b>Initial Step</b>	10 min. 95°C
<b>50 Cycles</b> (as required)	0 sec. 95°C 10 sec. 60°C

Typical profile times for this kit on this system when using Uracil-N-glycosylase and labelled probes are:

<b>Initial Step</b>	2 min. 50°C
<b>Heat killing UNG</b>	10 min. 95°C
<b>50 Cycles</b> (as required)	0 sec. 95°C 10sec. 60°C
<b>Hold</b>	50°C forever

When using Sybr™ Green I, an annealing temperature for 0 secs and an extension temperature of 72°C for 5-10secs may be used.

#### FOR IN VITRO 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 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-transferable 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 US patent 5,436,134 and 5,658,751 and licensed to Eurogentec S.A. by Molecular Probes in the direct research field.

The use of certain fluorogenic probes in 5' nuclease assays is covered by US patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc. and by US patent 5,538,848, owned by The Perkin-Elmer Corporation. Purchase of this product does not provide a license to use this patented technology. A license must be obtained by contacting the Director of Licensing Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404 or the Licensing Department at Roche Molecular Systems Inc., 1145 Atlantic Avenue, Alameda, CA 94501