

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 Master Mix - 3mM**

**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-35LC3**

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 Mastermix at -15 to -25°C in a constant temperature freezer.

## Kit Contents

The Lithos qPCR™ HotStart Mastermix contains enough PCR reagents for up to 350 - 20µl reactions.

| Reagent                                     | Volume | Description   |
|---|--------|---|
| 2X Master Mix<br>(Colour coded<br>as below) | 3.5ml  | seven tubes of Tris-HCl, BSA,<br>dNTP (inc. dUTP), Hot Goldstar<br>DNA polymerase and MgCl <sub>2</sub> ,<br>0.5ml each |
| MgCl <sub>2</sub><br>(Clear cap)            | 1.5ml  | one tube of 25mM MgCl <sub>2</sub>  |
| Sybr™ Green I<br>(Amber Cap)                | 5µl    | one tube of a 1,000 fold (1/10)<br>stock solution of Sybr™ Green I  |
| DMSO<br>(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 Mastermix.

### **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 Mastermix 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><br>(as required) | 0 sec. 95°C<br>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><br>(as required) | 0 sec. 95°C<br>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.

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