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

## **Lithos qPCR™ HotStart Master Mix - 2mM**

**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-35LC2**

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 (Colour coded as below)	3.5ml	seven tubes of Tris-HCl, BSA, 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 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> (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.

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