

HotGoldStar Mix

PK-0073-02 • PK-0073-02SA

Description

The ready to use 2x HotGoldStar Mix contains HotGoldStar *Taq* DNA polymerase, dNTPs, MgCl₂ and buffer. To prepare amplification, only add your primers, template DNA and water to the Mix.

Without activation, HotGoldStar *Taq* DNA polymerase completely lacks any activity below 74°C (“HotStart” activity avoids non specific priming at low temperature.)

Package contents

Reagent	Volume	Description
HotGoldStar Mix PK-0073-02 PK-0073-02SA	5 x 1ml 1ml	2 X PCR Mix: HotGoldstar, dNTPs, MgCl ₂ , buffer
MgCl ₂ 25 mM	1 ml	Additional 25 mM MgCl ₂ for optimization if needed (See MgCl ₂ optimization table)

Shipping conditions

Shipped on dry ice.

Storage conditions and stability

HotGoldStar Mix can be stored at -20 °C (in a constant temperature freezer) for 24 months or at 4°C for 3 months. Do not repeat more than 10 freeze/thaw cycles.

Quality control

Each lot is tested for activity by PCR. Using λ DNA as template we guarantee an amplification of at least 10⁵ fold.

Unit Definition

One unit of enzyme is defined as the amount required for incorporation of 10 nmoles of dNTPs into acid - insoluble material after 30 minutes amplification at 72 °C under the standard reaction conditions.

Reaction conditions

25 µl of the 2x HotGoldStar Mix diluted to a final volume of 50 µl will give a reaction medium that contains 1 units of HotGoldStar DNA polymerase, 200 µM dNTPs, 1.5 mM MgCl₂, 50 mM KCl, 15 mM Tris-HCl (pH 8.0 at 25°C), 0.01% (v/v) stabilizer.

Procedure

1. Thaw vial, mix and place on ice.
2. To 25 µl of HotGoldStar Mix, add 0.1 nmol of primers, template DNA* and H₂O to bring the total reaction volume to 50 µl.

Standard cycling conditions

<i>HotGoldStar Activation</i>	10 min at 95 °C
35 cycles:	
<i>Denaturation</i>	20 sec at 94 °C
<i>Annealing</i>	20 sec at 62 °C
<i>Elongation (suggested)</i>	30 sec at 72 °C

Time and temperature for denaturation and annealing steps depend on the type of machine and primers. We advise that you check primer design using a dedicated software.

No amplification will be observed without activation.

MgCl₂ optimization

MgCl ₂ final concentration	2× PCR Mix	MgCl ₂ (25 mM)	Primers, template, H ₂ O
1.5 mM	25 µl	0 µl	25 µl
2.0 mM	25 µl	1 µl	24 µl
2.5 mM	25 µl	2 µl	23 µl
3.0 mM	25 µl	3 µl	22 µl
3.5 mM	25 µl	4 µl	21 µl
4.0 mM	25 µl	5 µl	20 µl
4.5 mM	25 µl	6 µl	19 µl
5.0 mM	25 µl	7 µl	18 µl

For further information please contact our Customer Help Desk:

For Europe:

E-mail: info@eurogentec.com
Tel: +32 4 372 76 65

For USA:

E-mail: info.anaspec@eurogentec.com
Tel.: 510-791-9560 • toll-free: 800-452-5530

*Optimization of DNA input quantity may be required depending on the template DNA type and quality. We recommend determining the appropriate input range by testing undiluted template alongside 1:10 and 1:100 dilutions in parallel reactions. Excessive DNA input may inhibit PCR performance.