PCR using HotShot Diamond Mastermix

::: Guidelines

The HotShot Diamond Mastermix is delivered at a 2x concentration and contains reaction buffer (6mM $MgCl_2$) with 400µM dNTPs and Stabiliser. For PCR you use a final concentration of 1x.

::: Protocol

Take out desired volume of HotShot Diamond Mastermix (If using a 10ul reaction volume then you would use 5ul of the HotShot Diamond Mastermix.). Add DNA, primers, (fluorescence dye/probe) and make up to final volume with water.

A typical reaction mix would consist of the following:

Reagent	Volume to each well in final volume of 10ul	Final Concentration
HotShot Master Mix	5ul	1X
Forward Primer	1ul	0.25uM
Reverse Primer	1ul	0.25uM
Water	2ul	NA
DNA	1ul	10-20ng

HotShot Mastermix uses a Taq that has been mixed with an anti-Taq Antibody. To activate the Taq you will need to add a first step in your thermocycler protocol of 95°C for 5 minutes.

	Temperature	Time
Hold	95°C	5 minutes
30 - 45 cycles	94°C	As required.
	60°C –72°C	
	72°C	

Refreeze the rest of the HotShot Diamond Mastermix.
It can be frozen / thawed many times without loss of activity

P.O. Box 5563, Stourbridge, DY8 9AZ
Tel: 01384 444585 Fax: 01384 467980 Email: info@clentlifescience.co.uk