Biomiga Inc.

The Inventor of EZgene™ and ViraTrap™ Systems

Express Hi Fi DNA Polymerase (2 U/ µL)

Introduction

To ensure successful PCR using Express Hi Fi DNA Polymerase, the following guidelines are provided to cover standard PCR. PCR of templates with high secondary structure, low template concentrations require further optimization.

Components

Cat#	EHF DNA Polymerase	5x Buffer	20 mM $MgCl_2$	DMSO
EHF1101-02	2500 Units (1250 µL)	10x1200 µL	3 mL	1000 µL
EHF1101-01	500 Units (250 µL)	2x1200 µL	1 mL	500 µL

PCR Protocol

1. **Setup:** Assemble all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (95°C). All components should be mixed well and quickly spin down before start.

Component	20 µl Reaction	50 µl Reaction	Final Concentration
5X Express Hi Fi Buffer	4 µl	10 µl	1X
10 mM dNTPs	0.4 µl	1 µl	200 µM
10 µM fwd primer	1 µl	2.5 µl	0.5 µM
10 µM Rv Primer	1 µl	2.5 µl	0.5 µM
Template DNA	variable	variable	< 250 ng
DMSO (optional)	(0.6 µl)	(1.5 µl)	3%
Express Hi Fi DNA Polymerase	0.5 µl	1 µl	1.0 units/50 µl PCR
Nuclease-free water	to 20 µl	to 50 µl	

2. Notes: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

   Transfer PCR tubes from ice to a PCR machine with the block preheated to 98°C and begin thermocycling:

Thermocycling conditions for a routine PCR

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
25-35 Cycles	95°C 52-68°C 72°C	5-10 seconds 10-30 seconds 10 seconds per kb
Final Extension	72°C	8 minutes
Hold	4-8°C	

Important notes:

- Use of high-quality DNA templates greatly enhances the success of PCR. Recommended amounts of DNA template for a 50 µl reaction are as follows:

DNA	Amount
genomic	50 ng–250 ng
plasmid or viral	10 ng –50 ng

- The final concentration of each primer in a reaction using Express Hi Fi DNA Polymerase may be 0.2–1 µM.

- Mg^{++} is critical to achieve optimal activity with Express Hi Fi DNA Polymerase. The final Mg^{++} concentration in 1X Express Hi Fi HF Buffer is 1.5 mM. The optimal Mg^{++} concentration is affected by dNTP concentration; the template being used and supplements that are added to the reaction. This can also be affected by the presence of EDTA. Mg^{++} can be optimized in 0.2 mM increments using the $MgCl_2$ provided.

- Amplification of difficult template, such as GC-rich sequences or secondary structure, may be optimized by the presence of DMSO (included). A final concentration of 2% DMSO is recommended.

- The Express Hi Fi DNA Polymerase PCR products have blunt ends. Blunt-end cloning is recommended.