Ver: 1903

2×Express Hi Fi DNA Polymerase PCR Mix

（BW-EHF1102）

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Kit Contents

Catalog#	BW-EHF1102-00	BW-EHF1102-01	BW-EHF1102-02
2 × Express Hi Fi DNA Polymerase PCR Mix	100 μL	1.0 mL	5×1.0 mL
Nuclease-free water	100 μL	1.0 mL	5×1.0 mL
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Introduction

Express Hi Fi DNA Polymerase PCR Mix is a 2 × mix consisting of Express Hi Fi DNA Polymerase, deoxynucleotides and reaction buffer that has been optimized and includes $MgCl_2$. All that is required is the addition of template, primers and water.

Storage and Stability

Store all components at -20℃. All kit components are guaranteed for 12 months from the date of production.

Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each step.

Protocol

1. **Reaction setup:** We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (98°C). All components should be mixed and centrifuged prior to use. It is important to add 2×Express Hi Fi DNA Polymerase PCR Mix last in order to prevent any primer degradation caused by the 3′→ 5′ exonuclease activity. Please note that protocols with Express Hi Fi DNA Polymerase may differ from protocols with other standard polymerases. As such, conditions recommended below should be used for optimal performance.

Component	25 µL Reaction	50 µL Reaction	Final Concentration
10 µM Forward Primer	1 µL	1 µL	0.5 µM
10 µM Reverse Primer	1 µL	1 µL	0.5 µM
DMSO (optional)	(0.75 µL)	(1.5 µL)	(3%)
2 × Express Hi Fi DNA Polymerase PCR Mix	12.5 µL	25 µL	1 ×
Template DNA	variable	variable	< 250 ng
Nuclease-free water	to 25 µL	to 50 µL	

Note: 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	98°C	30 s
30-35 Cycles	98°C 45-72°C 72°C	5-10 s 10-30 s 10 s per kb
Final Extension	72°C	5-10 min
Hold	4°C	

2. **General guidelines**:

Template:

Use of high quality, purified 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–200 ng
plasmid or viral	1 pg–20 ng

3. If the template DNA is obtained from a cDNA synthesis reaction, the volume added should be less than 10% of the total reaction volume.

4. **Primers:**

Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%.

5. **Mg^{2+}, deoxynucleotides and additives:**

6. At 1× concentration, Express Hi Fi DNA Polymerase PCR Mix provides 1.5 mM $MgCl_2$ and 200 µM of each dNTP in the final reaction. Express Hi Fi DNA Polymerase PCR Mix cannot incorporate dUTP and is not recommended for use with uracil-containing primers or template.

Amplification of difficult targets, such as those with GC-rich sequences or secondary structure, may be improved by the presence of additives such as DMSO (included). A final concentration of 3% DMSO is recommended, although concentration can be optimized in 2% increments. It is important to note that if a high concentration of DMSO is used, the annealing temperature must be lowered as it decreases the primer Tm (1). Express Hi Fi DNA Polymerase PCR Mix is also compatible with other additives such as formamide or glycerol.

7. **Express Hi Fi DNA Polymerase Concentration:**

8. The concentration of Express Hi Fi DNA Polymerase in the Express Hi Fi DNA Polymerase PCR Mix has been optimized for best results under a wide range of conditions. If reactions are set up according to recommendations listed, the final concentration of Express Hi Fi DNA

Polymerase in the reaction is 2.5 U/50 μL or 1 U/20 μL.

9. **Denaturation:**

10. An initial denaturation of 30 seconds at 98°C is sufficient for most amplicons from pure DNA templates. Longer denaturation times can be used (up to 3 minutes) for templates that require it. During thermocycling, the denaturation step should be kept to a minimum. Typically, a 5–10 seconds denaturation at 98°C is recommended for most templates.

11. **Annealing:**

12. Annealing temperatures required for use with Express Hi Fi DNA Polymerase tend to be higher than with other PCR polymerases. Typically, primers greater than 20 nucleotides in length anneal for 10–30 seconds at 3℃ above the Tm of the lower Tm primer. If the primer length is less than 20 nucleotides, an annealing temperature equivalent to the Tm of the lower primer should be used. A temperature gradient can also be used to optimize the annealing temperature for each primer pair. For two-step cycling, the gradient can be set as high as the extension temperature.

    For high T_m primer pairs, two-step cycling without a separate annealing step can be used.

13. **Extension:**

14. The recommended extension temperature is 72℃. Extension times are dependent on amplicon length and complexity. Generally, an extension time of 15 seconds per kb can be used. For complex amplicons, such as genomic DNA, an extension time of 10-20 seconds per kb is recommended. Extension time can be increased to 30 seconds per kb for cDNA templates, if necessary.

15. **Cycle number:**

16. Generally, 30–35 cycles yields sufficient product.

17. **PCR product:**

18. The PCR products generated using 2×Express Hi Fi DNA Polymerase PCR Mix have blunt

ends. If cloning is the next step, then blunt-end cloning is recommended. If T/A-cloning is preferred, then DNA should be purified prior to A-addition, as Express Hi Fi DNA Polymerase will degrade any overhangs generated..

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.

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