Ver: 1903

Express High Fidelity DNA Polymerase

（BW-EHF1101）

Contents

Kit Contents

Catalog#	BW-EHF1101-00/10	BW-EHF1101-01/11	BW-EHF1101-02/12	BW-EHF1101-03/13
EHF DNA Polymerase	50 U (10 μL)	250 U (50 μL)	500 U (100 μL)	6×500 U (600 μL)
5 x Express Hi Fi PCR Buffer(with Mg^{2+})	400 μL	2000 μL	4×1000 μL	24×1000 μL
10 mM dNTPs	-/40 μL	-/200 μL	-/400 μL	-/2400 μL
Nuclease-free water	2mL	8 mL	16 mL	96 mL
DMSO	70 μL	350 μL	700 μL	4200 μL
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Introduction

The following guidelines are provided to ensure successful PCR using Express High Fidelity DNA Polymerase (Express Hi Fi DNA Polymerase). These guidelines cover routine PCR. Amplification of templates with high GC content, high secondary structure, low template concentrations or long amplicons may require further optimization.

Storage and Stability

Store all components at -20℃ except for DMSO (store at room temperature). 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:** Assemble all reaction components on ice and quickly transfer 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 Express Hi Fi DNA Polymerase 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	**20 µL Reaction**	**50 µL Reaction**	**Final Concentration**
Nuclease-free water	to 20 µL	to 50 µL	
5 x Express Hi Fi PCR Buffer (with Mg^{2+})	4 µL	10 µL	1×
10 mM dNTPs	0.4 µL	1 µL	200 µM
10 µM Forward Primer	1 µL	2.5 µL	0.5 µM
10 µM Reverse 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	0.2 µL	0.5 µL	2.5 U/50 µL PCR

Note: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary.

2. 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℃	30 s
30-35 Cycles	98℃ 45-72℃ 72℃	5-10 s 10-30 s 10-15 s per kb
Final Extension	72℃	5-10 min
Hold	4-10℃	

3. **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–250 ng
plasmid or viral	1 pg–20 ng

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

5. Primers:

Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as Primer 3 can be used to design or analyze primers. The final concentration of each primer in a reaction using Express Hi Fi DNA Polymerase may be 0.2–1 µM, while 0.5 µM is recommended.

6. **Mg^{2+} and additives:**

7. Mg^{2+} is critical to achieve optimal activity with Express Hi Fi DNA Polymerase. Excessive Mg^{2+} can prevent full denaturation of DNA as well as cause non-specific binding of primers. The optimal Mg^{2+} 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 chelators (e.g. EDTA). Mg^{2+} can be optimized in 0.5 mM increments using the $MgCl_2$

provided.

8. 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 . Express Hi Fi DNA polymerase is also compatible with other additives such as formamide or glycerol.

9. **Deoxynucleotides:**

10. The final concentration of dNTPs is typically 200 μM of each deoxynucleotide.

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

12. We generally recommend using Express Hi Fi DNA Polymerase at a concentration of 50 U/mL (2.5 U/50 μL reaction). However, the optimal concentration of Express Hi Fi DNA Polymerase may vary from 10–50 U/ml (0.5–2.5 U/50 μL reaction) depending on amplicon length and difficulty. Do not exceed 2.5 U/50 μL reaction, especially for amplicons longer than 5 kb.

13. **Buffers:**

14. 5×Express Hi Fi PCR Buffer (with Mg^{2+}) is provided with the enzyme. It is recommended as the default buffer for high-fidelity amplification.

15. **Denaturation:**

16. 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.

17. 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.

18. **Annealing:**

19. 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°C 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.

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

21. **Extension:**

22. The recommended extension temperature is 72°C. 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 30 seconds per kb is recommended. Extension time can be increased to 40 seconds per kb for cDNA templates, if necessary.

23. **Cycle number:**

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

25. Two-Step PCR:

26. When primers with annealing temperatures ≥ 72°C are used, a Two-Step thermocycling protocol is recommended.

27. Thermocycling conditions for a routine Two-Step PCR:

Step	**Temp**	**Time**
Initial Denaturation	98℃	30 s
30-35 Cycles	98℃ 72℃	5-10 s 15-30 s per kb
Final Extension	72℃	5-10 min
Hold	4-10℃	

28. **PCR product:**

29. The PCR products generated using EHF DNA Pol have blunt ends; if cloning is the next step, then blunt-end cloning is recommended. If TA-cloning is preferred, then DNA should be purified prior to A-addition, as EHF DNA Pol 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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