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DATA SHEET

Version: 2
Revision date: 05/06/2023

1. Identification

Product name **FastPANGEA™ High Fidelity DNA Polymerase**
(2 U/µL) 100 units

Cat. No **P0032**

2. Description

FastPANGEA™ High Fidelity DNA Polymerase is a second-generation High-fidelity DNA Polymerase that offers extreme performance for all PCR applications. It generates long templates with an accuracy and speed previously unattainable with other thermostable DNA polymerases. The error rate of FastPANGEA™ DNA polymerase is at least 50-fold lower than a normal Taq DNA Polymerase.

It possesses the 5'→ 3´ DNA polymerase activity, 3´→ 5´ exonuclease activity and it generates PCR products with blunt ends. It is also suitable for amplification of long amplicons such as 10-15 kb of genomic DNA.

3. Composition

Item	Quantity
FastPANGEA™ High Fidelity DNA Polymerase	100U
Buffer UNI (5x)	1.5 mL
DMSO (100%)	100 µL
MgCl2 (25 mM)	100 µL

4. Features

- **High Fidelity**
- **Robust Reactions:** maximal success with minimal optimization.
- **High Speed:** extension times are 15-30 seconds/kb.
- **High Yield**: increased product yield using minimal amount of enzyme
- **Versatile:** Can be used for routine PCR as well as long or difficult templates.

Quality Certifications:

- Functionally tested in PCR.
- Undetected bacterial DNA (by PCR)

5. Storage specifications

Store at -20°C.

6. Applications

- PCR-Cloning: highly recommended for cloning into pSpark® DNA cloning vectors.
- Primers extension.
- Long or difficult amplification.
- High-Throughput PCR.

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7. Further information

Product Use Limitations

This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer

The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handling or from contact with the above product.

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Before you start

Carefully centrifuge all tubes before opening to improve recovery. PCR reactions should be set up on ice. Prepare a mix for the appropriate number of samples to be amplified. The following protocol is recommended for a 20 µl reaction volume:

PCR PROTOCOL

1. **Assemble the following reagents in a thin-walled PCR tube.**

   Note!
   It is critical that the FastPANGEA - High Fidelity DNA polymerase is the last component added to the PCR mixture, since the enzyme exhibits 3'->5' exonuclease activity that can degrade primers in the absence of dNTPs.
   Carefully mix and centrifuge all tubes before opening to ensure homogeneity and improve recovery.

Component	Volume reaction 20 µL	Final concentration
Primer A	X µL	0.75 µM(1)
Primer B	X µL	0.75 µM(1)
Template DNA	X µL	20-50 ng DNA(2)
DMSO (optional)	0.6 µL	3% (3)
8 mM dNTPs	2 µL	0.8 mM (4)
Buffer UNI (5x)	4 µL	1X (5)
FastPANGEA™-Long PCR DNA Polymerase	0.2 µL	0.02U/µL (6)
$MgCl_2$ (25 mM)	2 µL	2.5mM
Nuclease-Free Water to a final volume of	20 µL	

(1) The recommendation for final primer concentration is 0.5 µM but it can be varied in a range of 0.2-1.0 µM if needed.
(2) For gDNA used 100-300 ng DNA.
(3) Addition of DMSO is recommended for GC-rich amplicons. If DMSO is added in the PCR reaction, Tm must be decreased about 3° C.
(4) For most of applications 200mM of each of dNTP's as final concentration is optimal. It's not necessary to optimize dNTP's concentration. dUTP or other dUTP derivatives should be used replacing dTTP in PCR reaction for "anti-contamination" PCR.
(5) 5X UNI Buffer provides very high reproducibility across the wide range of amplification conditions, including "fast-PCR" (reduced time of PCR reaction). 5X Uni Buffer contains 1.5mM Mg^{2+}, as the final concentration. In some cases, we recommend to optimize Mg concentration in the range 1.5-4.5mM
(6) An optimal amount of enzyme in 50µl reaction is 1U. In some cases, we recommend to optimize, depending on the length and complicity of amplified DNA sequence (increase up to 2U). For non-complex amplicons with the length less than 500bp and GC-content <60% amount of enzyme could be decreased up to 0.5 U per 50µl reaction.

2. **Mix reagents completely, and then transfer to a thermocycler.**

3. **Perform the following cycling conditions:**

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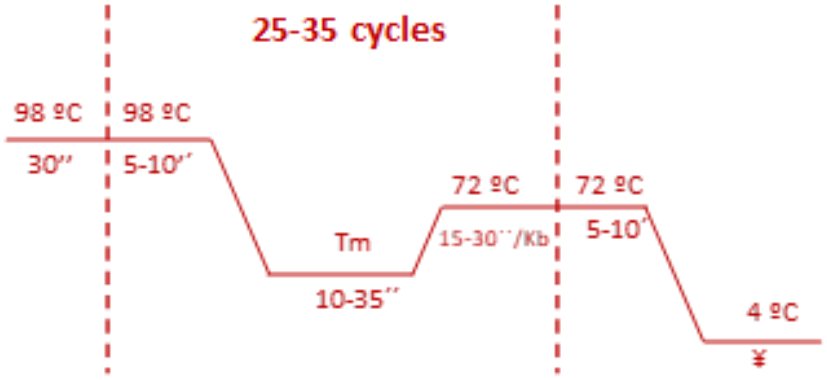

- As with all PCR reactions, conditions may need to be optimized to achieve maximum amplicon yield. You may be able to adjust your PCR conditions to optimize reaction.
- Genomic targets over 20kb may require additional optimization.
- As a basic rule, for primers > 20 nt, anneal for 10–30 seconds at a Tm +3 ˚C of the lower Tm primer. For primers ≤ 20 nt, use an annealing temperature equal to the Tm of the lower Tm primer. If necessary, use a temperature gradient to find the optimal annealing temperature for each template-primer pair combination.
- Extension time depends on amplicon length and complexity:
- For low complexity DNA (eg. Plasmid, lambda, or BAC DNA) use 15 s per Kb.
- For high complexity DNA (eg. gDNA) use 30s per kb. Do not exceed 1 min. per kb for amplicons that are <3 kb.