For research use only

ISO9001

HiPi DNA Polymerase

Product Description	Quantity	Cat. No.	Remarks
HiPi DNA Polymerase	500 unit	EBT-1002	5 unit/μl
	500 unit	EBT-1005	with 1 ml dNTP mix

Description

HiPi DNA Polymerase is a purified recombinant thermostable DNA polymerase from *Thermus aquaticus*. This enzyme is modified to increase specificity, fidelity, and performance in conventional PCR process. HiPi DNA polymerase is recommended for use in conventional PCR that the final products are smaller than 3 kbp. HiPi DNA Polymerase generates a 3′-dA overhang suitable for a TA cloning of PCR products.

Storage Buffer

5 unit/μl in 50 mM Tris-HCl, pH8.0, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.1% NP-40, 0.1% Tween 20, 50% Glycerol.

Unit Definition

One unit of enzyme catalyzes the incorporation of 10 nanomoles of deoxynucleotides into a polynucleotide fraction in 30 min at 72°C.

10x Reaction Buffer

500 mM Tris-HCl, pH9.0, 160 mM $(NH_4)_2SO_4$, 25 mM $MgCl_2$, 1% Tween 20, 1 mg/ml BSA.

QC tests

Activity, SDS-PAGE purity, performance tests, genomic DNA contamination test, confirmation test for the absence of endo and exonucleases.

Storage Condition

Store at -20°C.

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Standard Protocol

1. Prepare 50 μl PCR solution as follows:

PCR grade distilled water :	- μl
10x reaction buffer :	5 μl
10 mM dNTP mix (2.5 mM each) :	4 μl
Primer (10 pmol/μl) :	1 μl each
Template :	0.1-10 ng
HiPi DNA Polymerase :	0.2-0.5 μl (1-2.5 unit)

Adjust final vol. to 50 μl with PCR grade distilled water

*Note : Always, HiPi DNA Polymerase should be added last to the mixture

2. Set PCR cycling as follows :

Initial denature at 95°C : 3 min

		< 1 kbp	1-3 kpb
Denature	95°C	30 sec	30 sec
Anneal	Tm-4°C	30 sec	30 sec
Extend	72°C	45 sec	30-60 sec/kbp

25-40 PCR cycles

Trouble-Shooting

1. No products
   - Confirm your template is intact : Try another reaction with a result assured primer pair and templates
   - Be sure all the component are correctly added and working well : Sometimes low graded dNTP may inhibit the reaction, and degraded primers can result in low sized PCR fragments
2. Smear bands or smeared background
   - Reduce template concentration : High concentration of template can lead to smearing of PCR products. Generally, 1-10 ng of plasmid DNA and 10-100ng of genomic DNA are working well
   - Reduce enzyme concentration in the reaction
   - Increase annealing temperature
   - Set up a reaction mix on ice
3. Non-specific bands
   - Increase annealing temperature
   - Consider using PCR additives, like 1-2% DMSO and 0.5-1x Q buffer
   - Confirm specificity of your primers
4. Low yield
   - Increase enzyme concentration in the reaction
   - Increase PCR cycle number
   - Be sure appropriate concentration of your template is added