For research use only

ISO9001

HiPi Super DNA Polymerase

Product Description	Quantity	Cat. No.	Remarks
HiPi Super DNA Polymerase	250 unit	EBT-1301	5 unit/μl
	500 unit	EBT-1302	5 unit/μl

Description

Thermostable HiPi Super DNA Polymerase is suitable for an amplification of < 40 kb DNA fragments with a high fidelity and high specificity. It is highly effective in long PCR and multiplex PCR.

HiPi Super DNA Polymerase catalyzes the incorporation of nucleotides into duplex DNA in the 5′→3′ direction in the presence of Mg^{2+} at 70-80°C. HiPi Super DNA Polymerase is a mix of DNA polymerase showing 3′→5′ exonuclease (proof-reading) activity and Taq DNA Polymerase. Due to its high elongation rate, long sized products can be generated within relatively shorter running time compared to any other commercially available enzymes (10 kbp within 30 sec extension).

This enzyme is designed for a reliable amplification of long (up to 40 kbp for lambda DNA and 20 kbp for human genomic DNA) and complex targets with a robust yield and a high accuracy. HiPi Super DNA Polymerase generates a mixture of PCR products with blunt end and 3′-dA overhangs.

Storage Buffer

5 unit/μl in 20 mM Tris-HCl, pH8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20, and 50% Glycerol.

Unit Definition

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmole of dNTP into an acid-insoluble form in 30 min at 72℃.The reaction conditions are : 25 mM TAPS (pH9.3), 50 mM KCl, 2 mM $MgCl_2$, 1 mM β-mercaptoethanol, 200 mM each dNTPs, 100 μM [α^{-32}P]dCTP, 12.5 μg activated calf thymus DNA in a total volume of 50 μl.

10x Reaction Buffer

500 mM Tris-HCl, pH9.0 , 160 mM $(NH_4)_2SO_4$, 35 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 (2.5 mM each) :	4 μl
Primer (10 pmol/μl) :	1 μl each
Template :	0.1-10 ng
HiPi Super DNA Polymerase :	0.2-0.5 μl (1-2.5 unit)
Adjust final vol. to 50 μl with PCR grade distilled water	

* If you are amplifying a fragment larger than 2 kbp and with high GC content, add 5 μl of 10x Q buffer into the PCR mix. It will greatly improve reaction specificity. Q buffer is helpful for GC rich template and long template.

2. Set PCR cycling as follows :

Initial denature at 95°C : 3 min

		1-2 kbp	3-5 kpb	6-10 kbp	>10 kbp
Denature	95°C	5 sec	10 sec	20 sec	30 sec
Anneal	Tm-4°C	5 sec	10 sec	20 sec	30 sec
Extend	72°C	5 sec	10 sec	20 sec	30 sec/10 kbp

* Set 25-35 PCR cycles for effective amplification
* You can also use two step cycle for > 5 kbp amplification (denaturation at 95°C and annealing/extension at 68°C)

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 10-100 ng 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