For research use only

ISO9001

HiPi Super 5x PCR Master Mix

Product Description	Quantity	Cat. No.	Remarks
HiPi Super 5x PCR Master Mix	1 ml (250 reactions) 5 ml (1 ml x 5)	EBT-1604	4 μl/20 μl reaction

Description

HiPi Super 5x PCR Master Mix is a ready-to-use premix containing all the components essential for a PCR and agarose gel electrophoresis (DNA polymerase, dNTP, reaction buffer, glycerol, bromophenol blue, and stabilizer). PCR can be performed simply by adding primer pair and template.

As Master Mix is supplied as a 5x concentration format, users should adjust a final reaction to 1x (if final reaction volume is 20 μl, 4 μl of 5x Master Mix should be added). One unit of HiPi Super DNA Polymerase is contained in 4 μl of 5x Master Mix.

Thermostable HiPi Super DNA Polymerase is suitable for an amplification of < 40 kb DNA fragments with a high fidelity and 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.

QC tests

Performance tests, genomic DNA contamination test, confirmation test for the absence of endo and exonucleases.

Storage Condition

Store at -20°C.

HiPi Super 5x PCR Master Mix is stable for at least 2 years at recommended storage condition.

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Standard Protocol

1. Prepare 20 μl PCR solution as follows:

PCR grade distilled water :	– μl
HiPi Super 5x PCR Master Mix :	4 μl
Primer (10 pmol/μl) :	0.5 μl each
Template :	0.1-10 ng

Adjust final vol. to 20 μl with PCR grade distilled water

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/10kbp

* 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)

3. You can analyze PCR products by direct loading into agarose gel because PCR Master Mix contains glycerol and bromophenol blue (blue color) essential for a gel loading.

Trouble-Shooting

1. No products
   - Confirm your template is intact : Try another reaction with a result assured primer pair and templates
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
   - 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 or 0.5-1 M betaine
   - Confirm specificity of your primers
4. Low yield
   - Increase PCR cycle number
   - Be sure appropriate concentration of your template is added