For research use only ISO9001

HiPi Plus 5x PCR Master Mix

Product Description	Quantity	Cat. No.	Remarks
HiPi Plus 5x PCR Master Mix	1 ml (250 reactions) 5 ml (1 ml x 5)	EBT-1201	4 μl/20 μl reaction

Description

HiPi Plus 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 Plus DNA polymerase is contained in 4 μl of 5x PCR Master Mix.

HiPi Plus DNA Polymerase in Master Mix is suitable for a high fidelity amplification of DNA fragments. This enzyme is designed for a reliable amplification of long (up to 10 kbp for lambda DNA) and complex targets with a robust yield and high specificity. HiPi Plus 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 Plus 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 Plus 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 kbp	1-10 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

* Set 25-40 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
   - 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 is working well
   - Increase annealing temperature
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 PCR cycle number
   - Be sure appropriate concentration of your template is added