For research use only

ISO9001

Pfu Super 5x PCR Master Mix

Product Description	Quantity	Cat. No.	Remarks
Pfu Super 5x PCR Master Mix	1 ml (250 reactions) 5 ml (1 ml x 5)	EBT-1423	4 μl/20 μl reaction

Description

Pfu 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 Pfu Super DNA polymerase is contained in 4 μl of 5x Master Mix.

Pfu Super DNA Polymerase is suitable for a reliable amplification of long (up to 15 kbp) and complex targets with a robust yield and high accuracy. The error rate of Pfu Super DNA Polymerase is two times more less than that of Pfu DNA polymerase. The amplification efficiency (final yield) is about 2-3 times more than that of Pfu DNA polymerase. Furthermore, due to its high processivity, shorter running time is required compared to any other commercially available Pfu (5 kbp within 60 sec extension).

The amplified products by Pfu Super DNA polymerase can be used for a gene cloning with highly decreased error rate, and for a site-specific mutagenesis. Pfu Super DNA Polymerase, like any other polymerases showing proof-reading activity, generates a PCR product with blunt end.

Pfu Super DNA Polymerase exhibits 3′→5′ exonuclease (proof-reading) activity, but has no detectable 5′→3′ exonuclease activity.

QC tests

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

Storage Condition

Store at -20°C.

Pfu 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
Pfu 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	> 6kbp
Denature	95°C	10 sec	20 sec	30 sec
Anneal	Tm-4°C	10 sec	20 sec	30 sec
Extend	72°C	20 sec	60 sec	20 sec/1 kbp

* Set 25-35 PCR cycles for effective amplification
* You can also use two step cycle 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
5. Mutation is found
   - Increase initial template concentration
   - Reduce PCR cycle number