



For research use only

ISO9001

## Pfu Plus 5x PCR PreMix

Product Description	Quantity	Cat. No.	Remarks
Pfu Plus 5x PCR PreMix	96 reactions	EBT-1405	4 µl / 20 µl reaction
	5 x 96 reactions		

### Description

Pfu Plus 5x PCR PreMix is a ready-to-use premix pre-aliquoted in PCR tubes 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, template and PCR grade distilled water.

As PCR PreMix is supplied as a 5x concentration format in 4 µl volume within PCR tubes, users should adjust a final reaction volume to 20 µl by adding primers, template DNAs and distilled water. One unit of Pfu Plus DNA Polymerase is contained in 4 µl of 5x PCR PreMix.

Pfu Plus DNA Polymerase is suitable for a reliable amplification of long (up to 12 kbp) and complex targets with a robust yield and high accuracy. Although the error rate is slightly lower than that of Pfu DNA Polymerase, the amplification efficiency is about 3–5 times more. The amplified products by Pfu Plus DNA Polymerase can be used for a cloning of your genes with decreased error rate, and for a site-specific mutagenesis. Pfu Plus DNA Polymerase, like any other polymerases showing proof-reading activity, generates a PCR product with blunt end.

Pfu Plus 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 Plus 5x PCR PreMix is stable for at least 2 years at recommended storage condition.

### Standard Protocol

1. Prepare 20 µl PCR solution as follows:

5x PreMix in PCR tube :	4 µl
Primer (10 pmol/µl) :	0.5 µl each
Template :	0.1–10 ng
PCR grade distilled water :	– µl

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 kbp	> 6kbp
Denature	95°C	20 sec	20 sec	30 sec
Anneal	Tm-4°C	20 sec	20 sec	30 sec
Extend	72°C	30 sec	30 sec/kbp	30–60 sec/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 PreMix 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