WizPure™ Pfu 2X Master

- W1451 **1 ml**
- W1451-8 **8 X 1 ml**

Description

WizPure™ Pfu 2X Master is ready-to-use Pfu DNA polymerase pre-mixes are the innovation for convenience of your routine PCR as you just have to add your template DNA besides your primers & put the tubes for amplifications.
WizPure™ Pfu 2X Master is an optimized, ready-to-use PCR mixture of Pfu DNA Polymerase, Reaction buffer contains $MgCl_2$, dNTPs, enhancer and stabilizer. This Pfu 2X Master contains all components for PCR, except DNA template and primers. The mixture is suitable for amplification of most of the DNA templates.
In addition to 5´ to 3´ DNA polymerase activity, Pfu DNA Polymerase also possesses 3´ to 5´ exonuclease (proofreading) activity. Pfu DNA Polymerase exhibits the lowest error rate of any thermostable DNA polymerase studied, is even up to ten fold more accurate than normal Taq DNA polymerase. Consequently, Pfu DNA Polymerase is useful for polymerization reactions requiring high-fidelity synthesis.
PCR reactions can be directly loaded onto an agarose gel without the additional need of loading buffer and dyes.

Kit Contents

Contents	W1451	W1451-8
WizPure™ Pfu 2X Master	1 ml	8 X 1 ml

Applications

- High-fidelity PCR and primer-extension reactions
- Generation of PCR products for cloning and expression.
- PCR cloning and blunt-end amplification product generation
- RT-PCR for cDNA cloning and expression
- Site-directed mutagenesis
- Blunt-end PCR cloning

Storage Conditions

Store all components at -20ºC in a non-frost-free freezer.

Note

Do not contaminate the WizPure™ Pfu 2X Master with primers and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

Quality Control

No endonuclease activity, nicking activity, exonuclease activity, or priming activity has been detected.

Quality Authorized by : Jamie Ahn 

Protocol

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the Pfu 2X Master mix. For multiple reactions, scale-up volume of reaction components proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

1. Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.
2. Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.
3. The following table shows recommended component volumes:

Reaction Conditions

Component	20 µl reaction	Final Conc.
Pfu 2X Master	10 µl	1X
10µM Forward Primer	0.2~2.0 µl	0.1~1.0 µM
10µM Reverse Primer	0.2~2.0 µl	0.1~1.0 µM
Template DNA	≥ 1 µl	as needed
Water, RNase-Free	up to 20 µl	NA

NOTE: In general, use greater than 0.5 µM primers for sensitivity and less than 0.5 µM for specificity.
NOTE: Recommended amount of template per PCR reaction:

- < 50 ng plasmid or
- < 500~1000ng genomic DNA or
- 2µl of a 100µl single plaque eluate or
- one single bacterial colony

4. Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.
   (Optional) Overlay reactions with one-half volume PCR-grade mineral oil when not using heated lid on thermal cycler.
5. Transfer tubes into a PCR instrument and run as following table.

PCR Conditions

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	5 min.	1
Denature	95	30 ~ 60 sec.	25 ~ 40
Anneal	50 ~ 65	30 ~ 60 sec.	
Extend	72	30 ~ 60 sec.	
Final Extension	72	5 min.	1

IMPORTANT: Annealing temperature should be 2-6ºC lower than the primer melting temperature. Elongation time should be ~1 min/1 kb.

NOTE: Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

6. After cycling, maintain the reactions at 4°C or store at -20°C until ready for analysis.

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