

DESCRIPTION

WizPure™ FX-PCR 2X Master is a ready-to-use and complete system for rapid, consistent, and accurate amplification of long PCR products (>5~30 kb). This kit optimized for PCR amplification of genomic DNA templates up to 20 kb and lambda DNA up to 30 kb. With its enhanced processivity, yield, speed and excellent 3'→5' exonuclease and 3'→5' proofreading activity, this enzyme is able to consistently deliver accurate and reliable amplification of long templates. This product is the ideal choice for long DNA templates unable to be amplified in conventional PCR, and is highly suitable for multiple downstream applications including complex cloning and genotyping experiments. The PCR product amplified with this mixture has one A added at 3'-end, so the product can be directly used for TA cloning.

The kit already contained loading dye, therefore the PCR reactions can be directly loaded onto an agarose gel without the additional need of loading buffer and dyes.

KIT CONTENTS

Contents	W1461	W1461-5
WizPure™ FX-PCR 2X Master	1 ml	5 X 1 ml

APPLICATIONS

- Long range PCR
- High-fidelity PCR and primer-extension reactions
- Genotyping
- Library construction
- High GC amplification
- Next-generation DNA sequencing
- TA cloning

STORAGE CONDITIONS

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

NOTE

Do not contaminate the WizPure™ FX-PCR 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 ANALYSIS:

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizPure™ FX-PCR 2X Master kit is tested against predetermined specifications to ensure consistent product quality.

Quality Authorized by : Jang Min 

ORDERING INFORMATION

Product	Cat No.	Package
CrystalMix™ HS-PCR	W1461	1 ml
	W1461-5	5 x 1ml

PROTOCOL

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the FX-PCR 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:

Component	20 µl reaction	Final Conc.
FX-PCR 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.

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	1 min/kb	
Final Extension	72	5 min.	1




IMPORTANT: Annealing temperature should be 2-6°C 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.

Technical Support



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