WizPure™ HS-PCR 2X Master (UDG)

(For preventing carryover-contamination)

- W1412 **1 ml**
- W1412-8 **8 X 1 ml**

Description

WizPure™ HS-PCR 2X Master (UDG) is ready-to-use Hot-start PCR pre-mixes are the innovation for convenience of your routine PCR. The HS-PCR 2X Master contains an antibody-mediated hot-start Taq DNA Polymerase, $MgCl_2$, dATP, dCTP, dGTP, dUTP, Uracil DNA Glycosylase (UDG), enhancer and stabilizer.

UDG and dUTP are included in the mixture to prevent the reamplification of carryover PCR products between reactions. dUTP in the mix ensures that any amplified DNA will contain uracil. UDG removes uracil residues from single- or double-stranded DNA, preventing dU-containing DNA from serving as template in future PCRs.

The mixture is suitable for amplification of most of the DNA templates and highly processive 5′→3′ DNA polymerase that lacks 3′→5′ exonuclease activity and lacks a 3′→5′ proofreading function. PCR reactions can be directly loaded onto an agarose gel without the additional need of loading buffer and dyes.

Kit Contents

Contents	W1412	W1412-8
WizPure™ HS-PCR 2X Master (UDG)	1 ml	8 X 1 ml

Applications

- High through-put PCR
- Hot-start PCR
- Routine diagnostic PCR requiring high reproducibility
- DNA sequencing template preparation

Storage Conditions

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

Note

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

Preventing Template Carryover-Contamination

Due to the high sensitivity of PCR it is a risk that reaction may be contaminated with the products of previous runs. To minimize this risk, both dUTP and UDG is included in the HS-PCR 2X Master (UDG) mix mix to prevent PCR products from becoming source of contamination.

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 HS-PCR 2X Master (UDG) 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.
HS-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 ~ 5 µl	< 250 ng
Water, RNase-Free	up to 20 µl	

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
UDG Treatment	50	2 min.	1
Initial Denaturation	95	5 min.	1
Denature	95	10 ~ 60 sec.	25 ~ 40
Anneal	50~65	10 ~ 60 sec.	
Extend	72	60 sec./kb	
Final Extension	72	5 min.	1

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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