

## DESCRIPTION

CrystalMix™ HS-PCR kit contained all the reagents necessary for successful routine hot-start PCR in a convenient individually aliquot in an 8-strip PCR tube. CrystalMix™ HS-PCR kit is an economical, highly efficient, ready-to-use, and room temperature stable format. There is no need for freezing, thawing steps, or pipetting on ice, so minimized the risk of human errors and contaminations.

CrystalMix™ HS-PCR kit processive, 5'→3' DNA polymerase and lacks a 3'→5' proofreading function, therefore the amplification products are compatible with TA cloning. CrystalMix™ HS-PCR kit yields excellent and consistent results in routine hot-start PCR reactions as well as high-throughput PCR genotyping, colony PCR, RT-PCR, and PCR cloning. CrystalMix™ HS-PCR kit contained blue loading dye, therefore the PCR product can be directly loaded onto an agarose gel without the additional need of loading buffer and dyes. CrystalMix™ HS-PCR kit without gel loading dyes is also available for applications when loading dyes are undesired.

## APPLICATIONS

CrystalMix™ HS-PCR kit is suitable and tested for amplification of genomic targets ranging from 100 bp to 4 kb and of episomal targets (lambda phage; plasmids) up to 10 kb under various reaction conditions.

- High through-put PCR
- Routine diagnostic PCR requiring high reproducibility
- DNA sequencing template preparation
- Point-of-care Molecular diagnostics

## STORAGE CONDITIONS

- Store at below 25°C in the airtight pouch with the desiccant.
- Once opened, completely reseal the pouch with zipper.
- In high humidity environments, store unopened and resealed pouches in a desiccator to maximize product lifetime.
- Do not use once the cone-shape mix shrinks as dot-form. It damaged by re-hydration.

## NOTE

Do not contaminate the CrystalMix™ HS-PCR kit with primers and template DNA used in individual reactions.

## QUALITY CONTROL

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

Quality Authorized by : Jamie Ahn



## PROTOCOL

Please read through the entire protocol before starting.

Use the required number of tubes and immediately put the remaining tubes in the pouch and seal with the zipper.

1. Prepare the CrystalMix tube on the PCR tube rack.
2. Add the reaction component to the CrystalMix tube as the following table shows recommended component volumes:

Reaction Conditions

Component	20 µl reaction	Final Conc.
CrystalMix™ HS-PCR Tube	1 tube	1X
10µM Forward Primer	0.25~2.5 µl	0.1~1.0 µM
10µM Reverse Primer	0.25~2.5 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
- < 500~1000 ng genomic DNA or
- 2 µl of a 100 µl single plaque eluate or
- one single bacterial colony

3. 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 a heated lid on a thermal cycler.
4. Transfer tubes on ice into a thermal cycler and the following table shows recommended cycling conditions:

PCR Conditions

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	5 min.	1
Denature	95	10 ~ 30 sec.	30 ~ 45
Anneal	50~65	10 ~ 30 sec.	
Extend	72	10 ~ 60 sec.	
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.

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