Tel : 86-10-56953015
Email : info@cwbiotech.com

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# 2×Flash HS PCR MasterMix (Dye)

**Cat. No. :** CW3007M (5 mL)
CW3007L (25 mL)
CW3007H (40 mL)

**Storage Conditions:** -20°C; For frequent uses, store at 2-8°C.

## Components

| Component | CW3007M 5 mL | CW3007L 25 mL | CW3007L 25 mL |
|---|---|---|---|
| 2×Flash HS PCR MasterMix (Dye) | 5×1 mL | 25×1 mL | 40×1 mL |
| $ddH_2O$ | 5×1 mL | 25×1 mL | 40×1 mL |

## Introduction

This product is a premixed system composed of Flash HotStart DNA Polymerase, $Mg^{2+}$, dNTPs, PCR stabilizers and enhancers at a concentration of 2×. The Flash HotStart DNA Polymerase is featured with fast extension rate (5-15 sec/kb) and fast HotStart, which significantly shorten the duration of PCR. Meanwhile, it efficiently removes the non-specification amplification and enables high yield of PCR with high fidelity. This product has been added with dye (blue), and electrophoresis gel detection can be performed directly after the PCR. Most of the amplified PCR products have an "A" base attached to the 3' ends, and therefore can be directly used for T/A cloning. It provides a broad application for all conventional PCR reactions, such as gene cloning and genotyping.

## Quality Control

No exogenous nuclease activity was detected; No host DNA was detected by PCR; Single copy genes in multiple genomes could be efficiently amplified.

## Protocol

The following is an example of a PCR reaction system and reaction conditions for amplifying a 1 kb fragment using human genomic DNA as the template. The actual operation should be based on the template, the primers, and the size of the target fragment.

1. PCR reaction system:

| Reagent | 50 μL | Time |
|---|---|---|
| 2×Flash HS PCR MasterMix | 25 μL | 1× |
| Forward Primer, 10 μM | 2 μL | 0.4 μM[1)] |
| Reverse Primer, 10 μM | 2 μL | 0.4 μM[1)] |
| DNA template | < 0.5 μg | < 10 ng/μL |
| $ddH_2O$ | Up to 50 μL | |



2. PCR reaction program:

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Denaturation | 98°C | 10 sec | 25-35[4)] |
| Annealing | 55-65°C2) | 10-15 sec | |
| Extension | 72°C | 5-15 sec/kb[3)] | |
| Hold | 4-12°C | | |

**Note:**

**1) The range of the final concentration of the primer is 0.1-1.0 μM. When the amplification efficiency is low, the concentration of the primer can be increased; when a non-specific reaction occurs, the concentration of the primer can be reduced, thereby optimizing the reaction.**

**2) In general, the annealing temperature is 5°C lower than the melting temperature (Tm) of the primers. When the desired amplification efficiency cannot be obtained, the annealing temperature is appropriately lowered; whennon-specific reactions occur, the annealing temperature is increased, thereby optimizing the reaction conditions.**

**3) The extension time should be set according to the size of the amplicon. Use 5-10 sec for amplicons less than 1.5 kb and 5-15 sec/kb for amplicons large than 1.5 kb. For complicated DNA templates, further increase the extension time to obtain the high amplification efficiency.**

**4) The number of cycles can be set according to the downstream application of the amplification product. If the number of cycles is too small, the amount of amplification is insufficient; if the number of cycles is too big, the probability of mismatch increases, and the non-specific background is severe. Therefore, the number of cycles should be reduced as much as possible yet ensuring the yield of the product.**