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## Product information

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# HMB Extraction-Free PCR Mastermix - Blood

**Catalog #:** B690019
**Size:** 100 reactions
**Storage:** -20°C

## Product Description:

This kit is suitable for EDTA- or ACD-anticoagulated fresh blood, as well as refrigerated (frozen) blood. It enables direct amplification PCR on animal whole blood samples without nucleic acid extraction. The 2X HyperMB Blood Direct PCR Master Mix is a ready-to-use mixture that contains all components except primers, templates, and probes. The kit includes a genetically engineered DNA Polymerase combination, along with factors that inhibit non-specific PCR amplification and enhance reaction amplification efficiency, thereby greatly reducing non-specific PCR amplification and contamination.

## Storage Method and Precautions:

Shipped frozen; store at -20°C. See the package for the validity period.

## Kit Components:

| Components | |
|---|---|
| **2X HyperMB Blood Direct PCR Master Mix** | 2 x 1.25ml |
| **Protocol** | 1 |

## Standard Operating Procedure:

**1.** Prepare the reaction system in a PCR tube according to the table below.

| Component | 50 µl System |
|---|---|
| **2X HyperMB Blood Direct PCR Master Mix** | 25 µl |
| **Forward Primer (10 µM)** | 2 µl |
| **Reverse Primer (10 µM)** | 2 µl |
| **Blood Sample** | X µl |
| **ddH2O** | Up to 50 µl |

- The optimal concentration range of whole blood template is 0.5%~20%. It is recommended to use 10% as the initial trial condition, i.e., add 5 µl of whole blood as the template in a 50 µl reaction system. Be careful to avoid aspirating blood clots. For dried blood spots stored on Whatman® filter paper cards, a circular paper disc with a blood stain of approximately 1 $mm^2$ can be taken; no pretreatment is required, and it can be directly placed into the PCR reaction solution for amplification.
- After the PCR reaction, it is recommended to centrifuge the reaction product at 4000 rpm (1000× g) for 1~3 min to precipitate blood cell debris, and then take the supernatant for downstream analysis.

PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY.
NOT INTENDED FOR HUMAN OR ANIMAL USE.

 

**2.** Prepare the reaction system in a PCR tube according to the table below.

| Temperature | Time | Number of Cycles |
|---|---|---|
| 95°C | 5 min | 1 cycle |
| 95°C | 30 s | 30-35 cycles |
| 60°C | 30 s | |
| 72°C | 30 s/kb | |
| 72°C | 10 min | 1 cycle |
| 12°C | ∞ | - |

- The annealing temperature can be adjusted according to the Tm value of the primers.

## PCR Product Analysis:

After the PCR reaction, it is recommended to centrifuge the reaction product at 4000 rpm (1000× g) for 1~3 min to precipitate blood cell debris, and then take the supernatant for downstream analysis. This step can eliminate the interference of various residual components in whole blood on electrophoresis detection, and is especially necessary for PCR products using high-concentration blood as the template. Generally, 30~35 µl of supernatant can be obtained from a 5 µl/50 µl (10%) reaction product, and 40~45 µl of supernatant can be obtained from a 3 µl/50 µl (5%) reaction product. If other analyses of the PCR product are required (e.g., restriction enzyme digestion, sequencing), the product should be diluted 2~4 times to reduce the interference of salts and other inhibitors in the reaction on subsequent procedures.

## Frequently Asked Questions (FAQs):

**1. No Amplification Band or Weak Band**

| Possible Causes | Solutions |
|---|---|
| Loss of reagent activity due to improper storage or long-term storage. | Use fresh reagents. |
| Blood contamination or insufficient blood input. | Replace with fresh blood or slightly increase the input amount. |
| Blood anticoagulant is heparin. | This product does not currently support direct PCR amplification of blood stored with heparin anticoagulant. However, you can try by increasing the extension time and using 0.5%~2% blood sample. |
| Insufficient number of PCR cycles. | Increase the number of PCR cycles; 35~40 cycles are recommended. Due to the complexity of the template, the PCR reaction requires 5~10 more cycles than when using purified DNA templates. |

**2. Non-specific amplification**

| Possible Causes | Solutions |
|---|---|
| Too low PCR annealing temperature, or too high number of cycles, primer concentration, or template concentration. | Increase the PCR annealing temperature, and decrease the number of PCR cycles, primer concentration, or template concentration. |
| PCR primer mismatch. | Redesign the PCR primers. |

 