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Product information

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HMB Extraction-Free PCR Mastermix - Mammalian Tissue & Cells

Catalog #: B690020
Size: 100 reactions
Storage: -20°C

Product Description:

This product is a direct PCR kit specifically designed for animal tissues and cells. It greatly simplifies the experimental workflow and improves research efficiency with the following advantages:

1. **Direct Amplification:** No cumbersome nucleic acid extraction steps are required. PCR amplification can be performed directly on tissue samples such as mouse tail, ear, or toe, which significantly shortens the experimental time.
2. **Cell Compatibility:** It is also suitable for cell samples, allowing direct PCR amplification. This simplifies the cell processing procedure and enhances experimental convenience.
3. **Sequencing-Friendly:** The PCR products are pure and can be directly used for Sangon sequencing analysis without additional purification steps, providing convenience for subsequent research.
4. **High-Quality Enzyme System:** Uses hot-start Taq DNA polymerase with high specificity and sensitivity, ensuring amplification efficiency and high-yield PCR products.
5. **Wide Applications:** Suitable for research fields such as transgenic identification and genotyping, providing a reliable tool for researchers.

The 2X HyperMB Taq PCR Master Mix Pro provided in this kit is a 2× concentration hot-start PCR reaction mixture. It contains all components required for PCR amplification except templates and primers, which greatly simplifies the operation process and reduces the risk of contamination. This master mix is available for separate purchase.

The 2X HyperMB Taq PCR Master Mix Pro provided in this kit is a 2× concentration hot-start PCR reaction mixture. It The provided primer premix, SOX21 Primer Mix (10 µM each), can amplify a 237 bp fragment from the upstream conserved sequence of the SOX21 gene in mammals and most vertebrates, and can be used as a positive control.

Storage Method and Precautions:

- Store HyperMB Lysis Buffer in this kit at 2~8°C. Store 2X HyperMB Taq PCR Master Mix Pro at -20°C, and avoid repeated freezing and thawing. See the package for the expiration date.
- HyperMB Lysis Buffer and 2X HyperMB Taq PCR Master Mix Pro contain irritating compounds. During operation, wear a lab coat and latex gloves to avoid contact with skin, eyes, and clothing, and prevent inhalation. In case of contact with skin or eyes, rinse immediately with water or normal saline; seek medical assistance if necessary.

Kit Components:

Components	
HyperMB Lysis Buffer	25 ml
HyperMB Lysis Enhancer	1 ml
2X HyperMB Taq PCR Master Mix Pro	2 x 1.25 ml
Protocol	1

Standard Operating Procedures:

Before each use, check the status of HyperMB Lysis Buffer. HyperMB Lysis Buffer may precipitate at low temperatures; **thoroughly vortex to mix well before use.**

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1. For Mouse Tissue Samples

Take approximately 1~3 mm^2 of fresh or frozen mouse ear/liver tissue, 1~3 mm of mouse tail, or 1~2 mouse toes (frozen samples should undergo as few freeze-thaw cycles as possible) and add it to a PCR tube or 1.5 ml centrifuge tube. Then, add 50~200 µl of HyperMB Lysis Buffer and 10 µl of HyperMB Lysis Enhancer to the tube, ensuring the tissue is completely immersed in the lysis mixture. Incubate at 50°C for 10 min in a PCR instrument or metal bath, followed by inactivation at 95°C for 5 min.

2. For Cell Samples

Take approximately 10^3~10^7 cells, add 10~100 µl of HyperMB Lysis Buffer and 10 µl of HyperMB Lysis Enhancer, and ensure the cells are completely immersed in the lysis mixture. Incubate at 50°C for 10 min in a PCR instrument or metal bath, followed by inactivation at 95°C for 5 min.

- Fresh animal tissues are recommended; excessive repeated freezing and thawing of frozen tissues will significantly reduce the effect.
- For mouse tissues, 50 µl of HyperMB Lysis Buffer is usually sufficient for genome release of most tissues. For tissues with large quantity or hard-to-lyse properties, increase the volume of lysis buffer.
- For cells, 10 µl of HyperMB Lysis Buffer can typically lyse 10^3~10^5 cells, and 100 µl of HyperMB Lysis Buffer can handle up to 10^7 cells. To increase PCR yield, use less lysis buffer to lyse more cells.
- The heat inactivation step is mandatory.• Lysis products can be stored at 4°C for no more than 7 days, or the supernatant can be directly used for subsequent PCR amplification. Prolonged storage will lead to genomic DNA fragmentation.

2. PCR Reaction System Preparation

Cool the lysis products to room temperature (or wait for the PCR instrument/metal bath to cool to room temperature), then prepare the amplification reaction system in a PCR tube according to the table below.

Component	50 µl System	20 µl System
Lysis Product	1~4 µl	1~2 µl
Forward Primer (10 µM)	2.5 µl	1 µl
Reverse Primer (10 µM)	2.5 µl	1 µl
2X HyperMB Taq PCR Master Mix Pro	25 µl	10 µl
ddH2O	Up to 50 µl	Up to 20 µl

3. PCR Amplification Program

Place the PCR tube in a PCR instrument and run the following amplification program:

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.

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Frequently Asked Questions (FAQs):

1. No Amplification Band or Weak Band

Possible Causes	Solutions
Improper storage or expired reagents.	Use fresh reagents.
Template degradation or fragmentation.	Reduce the storage time after lysis or replace with fresh mouse tissue.
Excessive addition of tissue lysis buffer.	Increase the reaction volume or reduce the volume of lysis buffer.
Improper storage or prolonged storage of the sample lysis mixture (leading to genomic fragmentation).	Lysis products can be stored at 4°C for 15 days; use freshly prepared lysis products for PCR as much as possible.
Inappropriate template addition amount.	The template amount should not exceed 4 µl per 50 µl system; excessive template will inhibit PCR amplification.
Inadequate number of PCR cycles.	Increase the number of PCR cycles; 35~40 cycles are recommended.

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 reduce the number of PCR cycles, primer concentration, or template concentration.
PCR primer mismatch.	Redesign the PCR primers.

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