BIO BASIC
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Product information

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HMB Extraction-Free PCR Mastermix - Plant

Catalog #: B690018
Size: 100 reactions
Storage: -20°C

Product Description:

This product is a PCR direct amplification kit specifically designed for plant tissues. With the following advantages, it greatly simplifies the experimental process and improves research efficiency:

1. **Direct Amplification:** Eliminates the cumbersome nucleic acid extraction step, enabling direct PCR amplification from plant tissue samples and significantly reducing experimental time.
2. **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.
3. **High-Quality Enzyme System:** Utilizes hot-start Taq DNA polymerase with high specificity and sensitivity, ensuring amplification efficiency and high-yield PCR products.
4. **Wide Application:** 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 2X concentration hot-start PCR reaction mixture, which contains all components required for PCR amplification except the template and primers. It greatly simplifies the operation process and reduces the risk of contamination, and can be purchased separately.

Storage Method and Precautions:

- HyperMB Lysis Buffer in the kit should be stored at 2~8°C.
- 2X HyperMB Taq PCR Master Mix Pro should be stored at -20°C, and repeated freeze-thaw cycles should be avoided.
- The expiration date is indicated on the package.

HyperMB Lysis Buffer and 2X HyperMB Taq PCR Master Mix Pro contain irritating compounds. During operation, wear a laboratory coat and latex gloves to avoid contact with skin, eyes, and clothing, and prevent inhalation through the nose or mouth. In case of contact with skin or eyes, rinse immediately with water or physiological 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 Extraction Protocol:

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

1. Sample Lysis

Add approximately 1-3 mm^2 of fresh or frozen plant tissue (plant leaves are recommended) into a 200 µl PCR tube. Add 50 µl of HyperMB Lysis Buffer and 10 µl of HyperMB Lysis Enhancer to ensure the tissue is completely immersed in the lysis mixture. Incubate at 50°C for 10 min in a PCR instrument or dry bath, followed by inactivation at 95°C for 5 min.

- Tender leaves are recommended for plant tissue; slightly cutting them into pieces or mashing them with a pipette tip in the PCR tube will yield better results.
- The volume of lysis buffer can be adjusted according to the tissue size. Usually, 20~50 µl of HyperMB Lysis Buffer is sufficient for genomic release from most tender tissues. For difficult-to-lyse tissues, the volume of lysis buffer can be increased, but should not exceed 100 µl.
- The heat inactivation step is mandatory.
- The lysis product should 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 cause genomic DNA fragmentation.

2. PCR Reaction System Preparation

Allow the lysis product to return to room temperature, or cool the PCR instrument/dry bath to room temperature. Prepare the amplification reaction system in a PCR tube as shown in 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	35-40 cycles
60°C	30 s	
72°C	30 s/kb	
72°C	10 min	1 cycle
12°C	∞	

- If the plant tissue is mashed, the number of cycles can be appropriately reduced to 30; 40 cycles are recommended for mature leaves.
- The annealing temperature can be adjusted according to the Tm value of the primers.

Frequently Asked Questions (FAQs):

1. No Amplification Band or Weak Band

Possible Causes	Solutions
Reagent activity loss due to improper storage or long-term storage.	Use fresh reagents.
Excessive addition of tissue lysis buffer.	Increase the reaction system volume or reduce the amount of lysis buffer.

Possible Causes	Solutions
Improper storage or prolonged storage of the sample lysis mixture, leading to genomic DNA degradation.	The lysis product can be stored at 4°C for 15 days; use freshly prepared lysis product for PCR whenever possible.
Inappropriate template addition amount.	The template amount should not exceed 4 µl per 50 µl system; excessive amount will inhibit PCR amplification.
Inadequate number of PCR cycles.	Increase the number of PCR cycles; 35~40 cycles are recommended. Due to the complex template, PCR reactions require 5~10 more cycles than those 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 reduce the number of PCR cycles, primer concentration, or template concentration.
PCR primer mismatch.	Redesign the PCR primers.