ARMS PCR Mix Ⅱ

#P4011b, 1 mL

Contents:

2x ARMS PCR Mix Ⅱ	1 mL
Nuclease-free Water	1 mL

Store at -20°C for 2 years.

For research use only.

In total 2 vials.

Description

ARMS PCR Mix Ⅱ is a 2x PCR Mix solution developed for ARMS PCR, containing antibody-modified hotstart Taq DNA polymerase, dNTPs, high ionic buffer and other essential components for PCR amplification (except templates and primers). When used, only templates and primers need to be added into the amplification system, greatly simplifying the operation process, shortening the operation time, and reducing pollution (less sampling times). The reaction system of ARMS PCR Mix Ⅱ is specially optimized to reduce the formation of amplification products by mismatched primers and significantly improve the specificity of PCR amplification. This product can be used for conventional PCR electrophoresis and Real-Time PCR for ARMS-PCR detection.

ARMS-PCR (Amplification Refractory Mutation System PCR) is a diagnostic technique used to detect a specific mutation or genetic variation in a DNA sample. This technique uses primers to amplify only mutant alleles and not wild type alleles. By targeting specific mutations, ARMS-PCR can be used to identify disease-causing mutations, monitor disease progression, and study population genetic variation. It is a highly sensitive and specific method, which is widely used in the field of genetic testing and personalized medicine.

Applications

- ARMS PCR
- High specificity PCR

Features

- Convenient: only primers and template DNA are added when prepare final PCR
- High specificity: hotstart Taq DNA Polymerase
- High stability: the performance is not easy to change
- High sensitivity: efficient detection of mutations

Basic PCR Protocol

All solutions should be thawed on ice, gently vortex and briefly centrifuge.

1. Add the following components to a sterile microcentrifuge tube sitting on ice or at room temperature:

Reagent	Quantity	Final concentration
2x ARMS PCR Mix II	25 μl	1x
Forward Primer	variable	0.4 μM - 1 μM
Reverse Primer	variable	0.4 μM - 1 μM
Template DNA	variable	>10ng
Water, nuclease-free	to 50 μl	–

2. Mix contents in the tube. Cap tubes and centrifuge briefly to collect the contents to the bottom.

When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 μl mineral oil.

3. **Perform PCR amplification:**

Routine PCR reference procedure：

Stage	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	3 min	1
Denaturation	95°C	30 sec	32
Annealing	60°C	30 sec	
Extension	72°C	30 sec	
Final Extension	72°C	5 min	1

Real-Time PCR reference procedure：

Stage	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	3 min	1
Denaturation	95°C	30 sec	40
Annealing	60°C	30 sec	
Extension	70°C[1]	30 sec	

[1] : fluorescence collection.

4. Maintain the reaction at 4°C. The samples can be stored at -20°C until use.

5. Analyze the amplification products by qPCR, agarose gel electrophoresis and Sanger sequencing.

Notes

1, Storage conditions: the product should be stored at -20°C, cryogenic storage, avoid multiple freeze-thaw.

2. Preparation of reaction system: mix 2x ARMS PCR Mix evenly with primers, sample DNA and H_2O. It is suggested to optimize the

proportion of reaction components according to the concentration of template DNA.

3, Reagent stability: after opening, 4°C is limited to a month's use period. The reaction effect is not guaranteed after the use period.

4. Temperature and time: Before the PCR reaction starts, please confirm the accurate temperature of the PCR instrument. The most common temperature range for PCR reactions is 55°C to 65°C. The optimal amplification time is usually 30 seconds to 1 minute. The appropriate number of cycles and extension times can be adjusted as needed.

5. Contain at least one positive control and one negative control.

6. PCR product detection: Various PCR product detection methods can be used, such as gel electrophoresis, Sanger sequencing and other methods.

7. Some considerations for primer design of ARMS PCR:

7.1 Primer length: primer should generally be between 18-25 base pairs. Too short primer is easy to cause non-specific amplification, while too long primer will affect the amplification efficiency.

7.2 Proportion of primers: In ARMS PCR reaction, external primers and internal primers should be used in strict accordance with a certain proportion. In general, the concentration of the external primer should be slightly higher than that of the internal primer.

7.3 Selection of reverse complementary regions: This means that in the gene sequence, two "external" primers and two "internal" primers are designed by taking the variable shear site (SNP) as the dividing line. The external primer matches the two DNA sequences near the SNP respectively, while the internal primer matches the DNA sequence in the middle of the amplified sequence (target DNA) and is as close as possible to the SNP.

7.4 Reduce the risk of non-specific amplification: When designing ARMS PCR primers, cross hybridization with non-target sequences should be avoided. This can be achieved by adjusting PCR reaction conditions, screening suitable template DNA and optimizing primer design.

7.5 Design of multiple ARMS PCR primers: Since different genes have different variable loci (SNP), it is necessary to design multiple ARMS PCR primers to amplify the variable loci in different genes.

7.6 Primer sequence purity: To ensure high primer sequence purity, especially in the case of multiple SNP sites, to avoid cross-contamination between SNPs.

7.7 Test verification: The specificity and sensitivity of ARMS PCR primers were verified by sequencing, gel electrophoresis and other methods to ensure the amplification of target DNA sequences.