TruePol 2X PCR Mix for Microbiome

Cat.No. : RK20720

Product Components

Components	Component Number	Size-1	Size-2
Truepol 2X PCR Mix for Microbiome	RM20389	1 mL	1 mL × 5

Product Description

The DNA polymerase used in Truepol 2X PCR Mix for Microbiome is high fidelity hot start PCR polymerase. This polymerase is a novel mutant enzyme similar to *Pyrococcus furiosus*, which contains a recombinant synthetic enhanced domain. This unique structure improves the fidelity and extension speed of amplification. It is an antibody-mediated polymerase significantly inhibiting non-specific amplifications at room temperature.

Truepol 2X PCR Mix for Microbiome is developed for microbial diversity detection, having high amplification efficiency for various types of samples. Certain specific components mixted in this reagent enhance inhibitor tolerance and hence to improve the sensitivity. Even there is large amount of host DNA as interference in samples, Truepol 2X PCR Mix for Microbiome ensures good amplification efficiency, significantly improving the success of microbial detection rate. Truepol 2X PCR Mix for Microbiome can be used for dealing with general and complex samples, such as human tissue, soil, feces, urine, air filter membrane, sand, etc.

Truepol 2X PCR Mix for Microbiome contains DNA polymerase, dNTP, and an optimized buffer system. User only needs to add primers and templates to perform amplification, thereby reducing pipetting operations, significantly controlling cross-contamination among samples.

Storage

-20℃

5´-3´exonuclease activity

No

3´-5´exonuclease activity

Yes

Product End

Blunt end

Operation Description

Standard Protocol

1. Prepare all reaction components on ice, and then quickly transfer the reaction mix to a thermocycler preheated to 98℃.
2. Pipe up and down or vortex to mix, and centrifuge to collect the reaction mix to the bottom of the tube before use.
3. Add Truepol 2X PCR Mix for Microbiome at the end to prevent primer degradation by its strong 3´-5´ exonuclease activity.

Note: The Truepol 2X PCR Mix for Microbiome requires special reaction conditions different from other polymerase protocols. Please refer to the recommended reaction conditions below for the better amplification yields.

Recommended PCR Reaction

Recommended Reaction Mix:

Component	50 μL Reaction	Final Concentration
Truepol 2X PCR Mix for Microbiome	25 μL	1X
Forward Primer (10 μM)	2 μL	0.2-0.4 μM
Reverse Primer (10 μM)	2 μL	0.2-0.4 μM
DNA Template*	1-4 μL	/
Nuclease-free Water	to 50 μL	N/A

* Note: A stock solution or dilution of the DNA obtained by the kit.

Recommended PCR Program:

Step	Temp	Time	Cycles
Initial Denaturation	98℃	45 s	1
Denaturation	98℃	10 s	25-27
Annealing	55℃	60 s	
Extension	72℃	30 s/kb	
Final Extension	72℃	5 min	1
Hold	4-12℃	∞	1

PCR Principles

1. Template

High-quality purified DNA templates will increase the success rate of PCR. In general, samples can be amplified with 10 ng DNA template.

Complex samples, such as those with high host nucleic acid content or high inhibitor content, need to be diluted and then amplified, which can significantly improve amplification efficiency.

2. Primers

The final concentration of each primer in a PCR reaction can be adjusted from 0.2-1 μM, typically 0.4 μM and up to 0.6 μM for complex samples.

3. Denaturation

For most purified DNA templates, predenaturation at 98°C for 45 s provides adequate denaturation. For complex templates, such as high GC sequences, it is recommended to extend the initial denaturation time to 3 min for adequate denaturation. The recommended denaturation condition for most DNA templates is 98℃ for 5-10 s.

4. Annealing

For microbial diversity amplification experiments, the recommended annealing temperature is 55 ℃, which can also be adjusted according to primers. Increasing the annealing temperature helps to improve the specificity of PCR amplification.

5. Extention

The recommended extension temperature is 72℃. The extension time depends on the length and complexity of the amplicon. It can generally be extended at 30 s/kb. For high-complexity amplicons, it is recommended to increase the extension time to 60 s/kb.

6. Cycles

To obtain enough yield of PCR products, 25-30 cycles are recommended.

Q & A

The 16S amplification of microorganisms involves many types of samples, such as soil, sludge, feces, wine mash, fermented finished products, milk, alveolar lavage fluid, bile, fermentation, bacterial fluid, etc.. Success rate of amplifying such samples is greatly affected by factors interfering purity of template DNA (such as containing impurities, salt ions, pigments, humic acid, etc.). The following are protocol recommendations for differernt types of samples:

1. Fecal samples and soil samples

The nucleic acids extracted from fecal samples and soil samples contain many inhibitors, which are easy to inhibit the amplification reaction, resulting in poor amplification or even failure. The general recommendations are as follows:

1.1 For the 50 uL reaction mix system, fecal and soil DNA were directly injected into 1-2 μL, and the number of cycles was set to 25-30; If the reaction system is reduced, the input can be reduced accordingly.

1.2 If amplification fails, please analyze quality of DNA template or PCR product by agarose gel electrophoresis. If the gel pattern of the template DNA shows low DNA content, the input can be increased; If there are neither target bands nor other bands, the amount of template DNA can be reduced for optimization attempts; If there are no obvious bands, but only obvious diffuse bands, you can choose to reduce the amount of template DNA input or dilute the DNA by 2-4 fold, and then taking 2-4 μL for amplification optimization.

1.3 The quality of DNA extracted from different kits varies, so it is necessary to optimize the amount of DNA template input according to the actual situation, and choose the best protocol for sample amplification.

2. Whole blood sample

Whole blood samples contain more inhibitors. Extracted DNA is low in quantity and quality, which easily leads to poor amplification or even no amplification. The general recommendations are as follows:

2.1 For the 50 uL reaction mix system, add blood DNA 1-2 μL, and set PCR cycles 25-30; If the reaction mix system is reduced to other volume, accordingly reduce the volume of DNA template.

2.2 If amplification fails, template DNA input optimization can be performed, such as reducing the DNA template input by 1x, increasing the DNA template input by 1x, or diluting DNA template by 2-fold and then taking 2-4 μL for amplification optimization.

3. Tissue samples

The DNA extracted from tissue samples will have a large amount of host DNA that directly affect the amplification results. The general recommendations are as follows:

3.1 For the 50 uL reaction system, add 2-4 μL tissue sample DNA, and and set PCR cycles 25-30; If the reaction system is reduced, the template input can be reduced accordingly.

4. Other samples

4.1 According to the volume of reaction mix system, starting with 1-2 μL DNA template, and set PCR cycle 25-30; If the reaction system is reduced, DNA template input can be reduced.

4.2 For cases where amplification fails or does not perform well, analyze sample's purity, and then refers to opitimizing recommendations of Fecal samples and soil samples, whole blood sample, and tissue sample.