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Animal Detection Probe Mixture (UNG)

Product Number:PCM58

Shipping and Storage

-20°C, if frequently used, can be stored at 2-8°C to avoid repeated freeze-thaw as much as possible.

Components

Component	PCM58 5mL
2×Animal Detection Probe Mixture (UNG)	5×1mL
ddH_2O	5×1mL

Description

Animal Detection Probe Mixture (UNG) is a specialized premix suitable for probe detection of African swine fever virus (ASFV), with a concentration of 2×,Contains novel antibody modified Taq DNA polymerase, PCR Buffer, dNTPs, Mg^{2+}, as well as enhancers and stabilizers, making it convenient and fast to use.This product can be compatible with single and multiple probe qPCR reaction systems, and can also be used for detecting genomic DNA target sequences and cDNA target sequences after RNA reverse transcription.

This product uses the dUTP-UNG anti pollution system, and dUTP is added during the preparation process of the PCR reaction system, resulting in the formation of amplification products containing dU bases.And this product can be eliminated by UNG enzyme treatment in the PCR system before the next PCR reaction. This effectively removes residual contamination of PCR products and greatly reduces false positives caused by amplification product contamination.The pre denaturation step of UNG enzyme in the PCR cycle can be inactivated, so it will not affect the formation of new dU based PCR products.

This product contains highly sensitive engineered DNA polymerase modified with antibodies, which can effectively reduce non-specific amplification generated by non-specific binding of primers and templates or primer dimers under room temperature conditions, while significantly improving detection sensitivity and amplification efficiency. The activation of the enzyme only needs to be incubated at 95°C for 30 seconds, greatly shortening the reaction time of PCR.The carefully optimized PCR buffer system effectively inhibits the production of non-specific products, significantly improving the amplification efficiency and sensitivity of PCR.

Notes

Before use, please gently mix the product upside down after it has completely melted, avoiding foaming as much as possible, and use after briefly centrifuging.Avoid repeated freeze-thaw of this product, as repeated freeze-thaw may cause a decrease in product performance. This product can be stored in a dark place at -20°C for long-term storage.If frequent use is required in the short term, it can be stored at 2-8°C. ROX dye is used to correct the fluorescence signal error generated between quantitative PCR wells, and this product does not contain ROX dye.

Protocol

1. PCR reaction system

Reagent	50μL reaction	25μL reaction	Final Conc.
2×Animal Detection Probe mixture (UNG)	25 μL	12.5 μL	1×
Forward Primer, 10 μM	1 μL	0.5 μL	0.2 μM[1)]
Reverse Primer, 10 μM	1 μL	0.5 μL	0.2 μM
Probe[2)]	1 μL	0.5 μL	0.2 μM
Template DNA[3)]	X μL	X μL	

50×ROX reference dye(optional)[4]	1	0.5	1×
ddH_2O	Up to 50 μL	Up to 25 μL	

Note:1)Usually, a primer final concentration of 0.2μM can yield good results, which can be used as a reference for setting the range from 0.1 to 1.0μM.In the case of low amplification efficiency, the concentration of primers can be increased; When non-specific reactions occur, the concentration of primers can be reduced to optimize the reaction system.

2)The final concentration of the probe used is related to the fluorescent quantitative PCR instrument used, the type of probe, and the type of fluorescent labeling substance. Please refer to the instrument manual or the specific usage requirements of each fluorescent probe for concentration adjustment during actual use.

3)The amount of DNA templates is usually based on 10-100ng genomic DNA or 1-10ng cDNA as a reference. Due to the different copy numbers of target genes contained in templates of different species, gradient dilution can be performed on the templates to determine the optimal template usage

4)The excitation optical systems of different instruments vary, choose to add 50×Low ROX or 50×High ROX based on the instrument used for fluorescence quantification

Instruments without adding ROX correction	Roche LightCycler 480，Roche LightCyler 96，Bio-rad iCyler iQ，iQ5，CFX96
Instruments that require Low ROX calibration	ABI Prism7500/7500 Fast，QuantStudio® 3 System，QuantStudio® 5 System，QuantStudio® 6 Flex System，QuantStudio® 7 Flex System，ViiA 7 system，Stratagene Mx3000/Mx3005P，Corbett Rotor Gene 3000 etc.
Instruments requiring High ROX correction	ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus etc.

2. PCR reaction condition

Step	Temperature	Time	cycles
UNG digestion	50℃	2 min	1
Pre denaturation	95℃	30 s[1)]	1
Denaturation	95℃	10 s	45
Annealing/Extension	60℃	20 s[2)]	

Note:1)The enzyme used in this product is activated under pre denaturation conditions of 95℃ and 30 seconds.Under this condition, most templates can perform well in de chaining. For templates with high GC content and complex secondary structures, the pre denaturation time can be extended to 1 minute to fully unwind the initial template. If the high-temperature treatment time is too long, it will affect the enzyme activity; For simple templates, pre denaturation for 20 seconds can also be used, and the optimal pre denaturation time can be determined based on the template situation.

2)It is recommended to use a two-step PCR reaction program, and the annealing temperature should be set at 58-64℃ as a reference range. When non-specific reactions occur, the annealing temperature can be increased.If good experimental results cannot be obtained due to the use of primers with lower Tm values or excessively long amplification products, a three-step PCR amplification can be attempted. The annealing temperature should be set within the range of 56℃ -64℃ as a reference.

The annealing extension time settings for several common instruments are as follows:

1. When using Roche, BioRad, Agilent, Hongshi, Dongsheng, and other companies' fluorescence quantitative PCR instruments, please set it at 20 seconds.
2. When using ABI 7000/7300/7500, please set it to 30 seconds.
3. The annealing/extension time can be set according to the use of different models of instruments and templates. Please follow the requirements of the instrument user manual for experimental operations.