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ASFV Identification qPCR Mix UNG plus

Product Number: PCM16

Shipping and Storage

-20±5℃, for frequent use, 2×ASFV Identification qPCR Buffer U$^+$(for ASFV) can be stored at 2-8℃ to avoid repeated freeze-thaw as much as possible.

Components

Component	PCM16 50000T
50×Taq DNA Polymerase U$^+$ (for ASFV)	25mL
2×ASFV Identification qPCR Buffer U$^+$	625mL
RNase-Free Water	1L

Description

ASFV Identification qPCR Mix UNG plus mainly contains 2 × ASFV Identification qPCR Buffer U$^+$(for ASFV)and 50×Taq DNA Polymerase U$^+$ (for ASFV),Probe based fluorescence quantitative rapid detection for African swine fever virus.

2×ASFV Identification qPCR Buffer U$^+$(for ASFV) mainly includes optimized buffer systems, dNTPs, Mg^{2+},K^+, enhancers, and stabilizers,50×Taq DNA Polymerase U$^+$ (for ASFV) includes Taq DNA Polymerase and Uracil-N-Glycosylase, among which Taq DNA Polymerase is a dual antibody blocked hot start enzyme with 5'-3' DNA polymerase activity and 5'-3' exonuclease activity, without 3'-5' exonuclease activity and thermal stability,Holding at 94℃ for 1 hour still maintains 50% activity, with an enzyme elongation rate of 2 kb/min. The blocking rate of polymerase activity reaches over 95% at temperatures below 55℃, effectively reducing non-specific amplification and ensuring high specificity and precise quantitative analysis of PCR results. The blocking antibody is inactivated during the denaturation stage without the need for special inactivation treatment;Uracil-N-Glycosylase has no activity against RNA and can catalyze the release of free uracil from single and double stranded DNA containing uracil, effectively clearing system contamination. This product has strong stress resistance in amplification of low concentration blood samples, saliva samples, etc.

Note

1. Before use, please gently mix it upside down and avoid foaming as much as possible. After briefly centrifuging, use it.
2. 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±5℃ for long-term storage. If frequent use is required in the short term, 2×ASFV Identification qPCR Buffer U$^+$(for ASFV) can be stored at 2-8℃.

Protocol

The following examples are the conventional PCR reaction system and reaction conditions. In practical operation, corresponding improvements and optimizations should be made based on different templates, primer structures, and target fragment sizes.

1. PCR reaction system

Reagent	25μl reaction system	Final Conc.
2×ASFV Identification qPCR Buffer U$^+$	12.5 μL	1×
50×Taq DNA Polymerase U$^+$ (for ASFV)	0.5 μL	1×
Forward Primer	0.25 μL	0.1 μM-0.5 μM
Reverse Primer	0.25 μL	0.1 μM-0.5 μM
Probe	0.05 μL	50 nM-250 nM

Template	X μL
RNase-Free Water	up to 25 μL

Note:1)Usually, a primer 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.

2. PCR reaction conditions

Step	Temperature	Time	
UNG digestion	37℃	2 min	
Pre denaturation	95℃	3 min	
denaturation	95℃	10 s	45cycles
Annealing/Extension[1)]	60℃	30 s	*Collect signals here

Note:The annealing temperature and time can be set according to the actual primers and probes.