Animal Detection U+ Probe Super Premix

QV114

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Instruction for Use

Version 1.1

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01/Product Description

Animal Detection U+ Probe Super Premix is a specialized master mix for probe qPCR to detectAfrican swine fever virus (ASFV). Only need to input extra primers, probes and templates into the reaction system. This master mix employs an upgraded hot-start Taq polymerase with a carefully optimized buffer to increase detection sensitivity for low-copy templates. This product incorporates a dUTP/UDG anti-contamination system, which works at room temperature to eliminate the contamination of DNA product carryover, ensuring the accuracy of the results. It is also compatible with fast program, shortening test time.

02/Components

Components	QV114-01 (400 rxns/25 μl reaction)	QV114-02 (800 rxns/25 μl reaction)
2 × Animal Detection U+ Probe Super Premix[a]	5 × 1 ml	10 ml
50 × ROX Reference Dye 1[b]	200 μl	400 μl
50 × ROX Reference Dye 2[b]	200 μl	400 μl

a. It includes dNTP/dUTP Mix, Mg^{2+}, hot-start Taq polymerase, heat-labile UDG, etc.

b. It is used to correct fluorescence signal errors between wells. Refer to the following table to choose ROX Reference Dye for different instruments:

No ROX Reference Dye is required	Bio-Rad CFX96, CFX384, iCycler iQ, iQ5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo4; Qiagen/Corbett Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000; Cepheid SmartCycler; Eppendorf Mastercycler ep realplex, realplex 2s; Illumina Eco qPCR; Roche Applied Science LightCycler 480; Thermo Scientific PikoReal Cycler.
Add ROX Reference Dye 1 (final concentration 1 ×)	Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOnePlus.
Add ROX Reference Dye 2 (final concentration 1 ×)	Applied Biosystems 7500, 7500 Fast, ViiA 7; Stratagene MX4000, MX3005P, MX3000P.

03/Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

04/Applications

It is suitable for probe-based qPCR detection of DNA template.

05/Notes

Please mix thoroughly before use. Avoid repeated freezing and thawing.

06/Experiment Process (Use ABI 7500 as a test model)

1. Prepare the following mixture in a qPCR tube

2 × Animal Detection U+ Probe Super Premix	12.5 μl
Primer 1 (10 μM)	0.5 μl
Primer 2 (10 μM)	0.5 μl
TaqMan Probe (10 μM)	0.5 μl
ROX Reference Dye 2	0.5 μl
Template DNA	5 μl
ddH_2O	5.5 μl

The amount of each component in the reaction system can be adjusted according to the following principles:

▲ The primer concentration can be adjusted within a final concentration 0.1 μM to 1.0 μM.

▲ The final probe concentration can be adjusted to 50 nM to 250 nM.

▲ Template can be adjusted to an appropriate volume.

2. Perform the qPCR reaction according to the following conditions

Standard Program

Stage 1	Contamination digestion	Rep: 1	37˚C	2 min
Stage 2	Initial-denaturation	Rep: 1	95˚C	30 sec
Stage 3	Cycles	Reps: 45	95˚C	10 sec
			60˚C	30 sec

Fast Program

Stage 1	Contamination digestion	Rep: 1	37˚C	2 min
Stage 2	Initial-denaturation	Rep: 1	95˚C	20 sec
Stage 3	Cycles	Reps: 45	95˚C	1 sec
			60˚C	20 sec*

* Please confirm if the Real Time PCR instrument is in support of rapid amplification cycles.

07/Precautions for primer design

1. The ideal primer length is 17 - 25 bp. Primers that are too short are likely to result in reduced amplification efficiency. Primers that are too long are more likely to generate higher structures. Both of them will interfere the accuracy of the quantitative results.
2. Control the GC content of the primers at 40% to 60%, and the optimum GC content is from 45% to 55%.
3. The Tm value of the primer should be greater than 60˚C. Primer Premier 5 is recommended to calculate the Tm value.
4. The overall distribution of A, G, C, and T in the primer should be as uniform as possible. Avoid using regions with high GC or TA content, especially at the 3' end. Regions with uneven GC content must be avoided.
5. Try to avoid structures with consecutive T/C or A/G when designing primers.
6. The last five bases at the 3' end of the primer must not contain more than two G or C.
7. Ensure that the forward or the reverse primer is as close as possible to the probe sequence, but does not have overlap regions with the probe sequence.

08/FAQ & Troubleshooting

◇ Abnormal amplification curves

① The amplification curve is not smooth: The signal is too weak and is generated after system correction. Increase template concentration and repeat the experiment.

② There is a break in the amplification curve or it goes downward: The template concentration is too high and the baseline endpoint is greater than C_T value. Reduce the baseline endpoint (C_T value -4) and repeat data analysis.

③ There is a sudden drop in an individual amplification curve: There are bubbles remaining in the reaction tube. Pay attention to centrifugation when processing samples and carefully check the reaction tube for any remaining bubbles before performing an amplification reaction.

◇ No amplification curve appears at the end of the reaction

① Insufficient number of reaction cycles: Typically the number of cycles is set to 45, but it must be noted that too many cycles can also add too many background signals and reduce the reliability of the data.

② Confirm whether a signal acquisition step is set up in the procedure: In a two-step amplification procedure, signal acquisition is typically set in the annealing and extension phase. In a three-step amplification procedure, signal acquisition should be set in the 72˚C extension phase.

③ Check if the primers are degraded: Primers that have not been used for a long time should be tested for integrity using PAGE electrophoresis before use in order to rule out the possibility of degradation.

④ Template concentration is too low: Lower the level of dilution and repeat the experiment. In general, samples with unknown concentration should be started at the highest concentration.

⑤ Template degradation: Prepare new template and repeat the experiment.

◇ C_T value appears too late

① Extremely low amplification efficiency: Optimize the reaction conditions, attempt a three-step amplification procedure, or re-design the primer.

② Template concentration is too low: Reduce the dilution factor and repeat the test. In general, samples with unknown concentration should be started at the highest concentration.

③ Template degradation: Prepare new template and repeat the test.

④ PCR products are too long: The recommended PCR product length is 80 bp to 150 bp.

⑤ PCR inhibitors are present in the solution: They are usually introduced along with the template. Increase the template dilution factor or prepare the template again and repeat the test.

◇ The negative control is amplified markedly

① The reaction solution is contaminated: Replace the mix, ddH_2O, primers and probe, then repeat the test.

◇ Standard curve linearity is poor for absolute quantification

① Sample loading error: Increase the template dilution factor and the loading volume of sample.

② Standard product degradation: Prepare the standard again and repeat the test.

③ Template concentration is too high: Increase the template dilution factor.

◇ Poor experiment repeatability

① The sample loading volume is inaccurate: Use a higher performance pipette; increase the template dilution factor, and increase the sample loading volume.

② There are large differences in the temperature control wells of the quantitative PCR instrument: Calibrate the instrument regularly.

③ The template concentration is too low: The lower the concentration of the template is, the worse the repeatability is. Lower the dilution level of the template or increase the sample loading volume.