Animal Detection U+ Probe Master Mix

QV110

Vazyme

Instruction for Use

Version 24.1

Vazyme

Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

Animal Detection U^{+} Probe Master Mix is a specialized master mix for probe qPCR to detect African swine fever virus (ASFV). Only need to add extra primers, probes and templates. This master mix utilizes an upgraded hot start *Taq* DNA polymerase with a carefully optimized buffer to increase detection sensitivity for low-concentration templates. It is compatible with fast program, reducing test time. In addition, the dUTP/UDG anti-contamination system is introduced in it, which can work at room temperature to eliminate the influence of amplification product contamination on qPCR and ensure the accuracy of results.

02/Components

Components	QV110-01 400 rxns (25 µl/rxn)	QV110-02 800 rxns (25 µl/rxn)
2 × Animal Detection U^{+} Probe Master Mix[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 DNA polymerase, Heat-labile UDG, etc.

b. It is used to correct the error of fluorescence signals between wells. Use 50 × ROX Reference Dye 1 for ABI 7900HT/7300 Real-Time PCR System and StepOnePlus; Use 50 × ROX Reference Dye 2 for ABI 7500, 7500 Fast Real-Time PCR System, and Stratagene Mx3000P. Don't use ROX for Roche and Bio-Rad Real-Time PCR instruments.

03/Storage

Store at -30 ~ -15°C and ship at ≤0°C.

04/Applications

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

05/Notes

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

06/Experiment Process (Using ABI 7500 as a test machine)

1. Prepare the following mixture in a qPCR tube

Components	Volume
2 × Animal Detection U⁺ Probe Master Mix	12.5 μl
Primer1 (10 μM)	0.5 μl
Primer2 (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 volume of each component in the reaction system can be adjusted according to the following principles:

▲ The final concentration of primer can be adjusted between 0.1 - 1.0 μM.

▲ The final concentration of TaqMan Probe can be adjusted between 50 - 250 nM.

▲ Template can be adjusted to an appropriate volume.

2. Run the qPCR program as follows

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*

* Signal Acquisition.

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/Primer Design Guidance

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 with the accuracy of quantitative results.
2. Control the GC content of the primers at 40% - 60%, and the optimum GC content is from 45% - 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 shape of amplification plot

① Rough amplification plot: The signal is too weak and generated after system correction. Increase template concentration and retry.

② Broken or downward amplification plot: 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 Amplification plot goes downward suddenly: 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 reaction.

◇ No amplification plot

① Insufficient number of reaction cycles: In general, the number of cycles is set to 40, but it should be noted that too many cycles will increase too many background signals and reduce the reliability of the data.

② Confirm whether the signal acquisition step is set up in the program: The two-step amplification program generally sets the signal acquisition at the annealing and extension stage, while the three-step amplification program should set the signal acquisition at the 72°C extension stage.

③ Confirm whether 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 Low template concentration: Reduce the dilution factor and repeat the test. In general, samples with unknown concentration should be started at the highest concentration.

⑤ Template degradation: Prepare new template and retry.

◇ C_T value appears too late

① Low amplification efficiency: Optimize the PCR system, then try the three-step amplification program or redesign the synthetic primers.

② Low template concentration: 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 retry.

④ Long PCR products: The recommended length of PCR products is 80 - 150 bp.

⑤ PCR inhibitors in the system: They are usually introduced along with the template. Increase the dilution factor or prepare new template and retry.

◇ Amplification observed in negative control

① Contaminated of reaction system: Replace with new enzymes, Buffer, ddH_2O, primers, and probes, and retry.

◇ 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 Inaccurate pipetting volume: Use higher performance pipette; increase the template dilution factor, and increase the sample loading volume.

② Differences in temperature control between wells in qPCR instrument: Calibrate the instrument regularly.

③ Low template concentration: The lower the template concentration, the worse the repeatability. Reduce the template dilution factor or increase the volume of sample addition.

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