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Vesicular Stomatitis Virus VSV-G Dye-Based Quantitative RT-PCR Kit

Product Number: DTK550

Shipping and Storage

Low temperature transportation, stored at -20℃, with a shelf life of 12 months. Positive controls need to be placed separately due to their tendency to contaminate other components. This product does not provide live samples as positive controls, only nucleic acid fragments as positive controls.

Component

Component	Specification	Nine hole box packaging
2×Probe qPCR MagicMix	500μL	Brown cover
10 × qRT-PCR enzyme mixture	100μL	Red cover
Fluorescent PCR specific template diluent	1mL	Green cover
Vesicular stomatitis virus VSV-G dye based qRT-PCR primer mixture	100μL	White cover
Vesicular stomatitis virus VSV-G dye qRT-PCR positive control (1 × 10E7 copy/μL)	50μL	Yellow cover

Description

Vesicular stomatitis is a zoonotic infectious disease caused by a virus. It is mainly manifested in the occurrence of blisters on the oral mucosa, occasionally on the hoof crown and interphalangeal skin, and the appearance of foam like things. Infected animals are the main source of transmission for this disease. The initial manifestation of diseased animals is an increase in body temperature, followed by the appearance of blisters in the mouth and hooves. After the blisters rupture, the wound is not easily healed.

Application

This product is developed based on the principle of dye based fluorescence quantitative PCR, specifically for the detection of vesicular stomatitis virus VSV-G. It has the following characteristics:

1. One stop shop, users do not need to prepare each ingredient separately, including primers and controls.
2. Primers designed based on the conserved gene sequence of the vesicular stomatitis virus VSV-G genome have good specificity.
3. Based on dye based qRT-PCR detection, the sensitivity is 10-100 times higher than conventional RT-PCR.
4. Using one tube qRT-PCR technology, RT and PCR are completed in one test tube, without the need for intermediate sample transfer, reducing operational errors and potential contamination.
5. This product is sufficient for 50 rounds of RT-PCR in a 20μL system and can only be used for scientific research, not for clinical use.

Specimen collection

Sample DNA.

Protocol

1. **Dilute the positive control**

   taking the 10 fold dilution of the 6 samples 10E1-10E6 as an example. Due to the high concentration of the standard, the following dilution operations must be performed in separate areas. To increase product stability and avoid the spread of infectious pathogens.

1.1. Mark 6 centrifuge tubes, namely 6, 5, 4, 3, 2, and 1.

1.2. Add 45μL of fluorescent PCR specific template diluent with a core gun tip, preferably using a core gun tip, the same below.

1.3. Add 5μL of 1 × 10E7 copy/μL positive control (provided by the reagent kit) to tube 6, shake thoroughly for 1 minute, and obtain 1 × 10E6 copy/μL standard curve sample. Put it on ice for later use.

1.4. Change the gun head and add 5μL of 1 × 10E6 copy/μL positive control (diluted in the previous step) to tube 5. Shake thoroughly for 1 minute to obtain a standard curve sample of 1 × 10E5 copy/μL. Put it on ice for later use.

1.5. Change the gun head and add 5μL of 1 × 10E5 copies/μL positive control (obtained from the previous dilution) to tube 4. Shake thoroughly for 1 minute to obtain a standard curve sample of 1 × 10E4 copies/μL. Put it on ice for later use.

1.6. Repeat the above operation until obtaining standard curve samples with 6 dilutions. Put it on ice for later use.

2. Preparation of Sample DNA

2.1. If there are N samples, N+2 extractions must be set, with the additional one being PC (positive control for sample preparation) and one being NC (negative control for sample preparation). A positive control can be prepared by adding a certain amount of water to the fourth sample (with a concentration of 1 × 10E4 copies/μL, where 10μL is equivalent to 100000 copies) in the positive control gradient dilution solution prepared in the previous step (the total volume after adding water is the same as the sample, and the sample volume depends on the requirements of the reagent kit used). Water can be used as a negative control for preparation.

2.2. Purification of RNA from N+2 samples using a self selected method, this kit is compatible with most viral RNA extraction kits on the market. We recommend using our company's RNA extraction kit for extraction.

3. Set up RT-PCR reaction (20 μL system, conducted in the sample preparation room)

3.1. If quantitative analysis is performed and only one repetition is made, label N+9 PCR tubes, of which N+2 are used for the N+2 samples obtained in the previous step, 1 is used for PCR negative control, and 6 are used for the standard curve. If qualitative analysis is performed and only one repetition is made, label N+4 PCR tubes, of which N+2 are used for the N+2 samples obtained in the previous step, 1 is used for PCR negative control, and 1 is used for PCR positive control (using the 4th positive control dilution as a template). The following only describes the steps of quantitative analysis, while qualitative analysis only reduces 6 standard curve reactions to 1, leaving the rest unchanged.

3.2. Add each component to the labeled tube according to the table below (this table only lists one repetition. The positive control is only set after the sample tube and negative control are set, and the positive control sample is added after all tubes are closed):

Ingredients/per tube	Sample tube N+2	PCR negative control tube	Standard curve sample tube (1-6 tubes)
2 × qRT-PCR buffer	10μL	10μL	10μL
Vesicular stomatitis virus VSV-G dye based qRT-PCR primer mixture	2μL	2μL	2μL
RNA template obtained from sample preparation (from step 6)	6μL	-	-
Dilute to obtain 6 positive controls (from step 4)	-	-	6μL (sample 1 to tube 1, sample 2 to tube 2...)
10 × qRT-PCR enzyme mixture	2μL	2μL	2μL

Note: Some qPCR instrument models require the addition of ROX dye calibration to eliminate the optical path difference between tubes and maintain consistency for more accurate experimental data.

Models that require the use of ROX dye I: ABI Prism7000, 7300, 7700, 7900HT, Step-One, Step-One Plus, Suggest a final concentration of 500nM.

Models that require the use of ROX dye II: ABI Prism 7500, 7500Fast, MJ Research's Chromo4 Opticon (II) Corbett Rotor Gene 3000, Suggest a final concentration of 50nM.

3.3. After installation, perform RT-PCR according to the following parameters (parameters may need to be optimized due to different instruments):

Process	Temperature	Time
RT (reverse transcription)	50℃	2 min
Pre denaturation	95℃	10 min
qPCR reaction (40 cycles)	95℃	15sec
	60℃	60 sec (collect fluorescence signals from SYBR channel)
Perform melting curve analysis according to the preset program of the instrument		

4. **Data processing**

4.1. If this reagent kit is used for quantitative detection, plot a standard curve with the log value of positive control concentration as the horizontal axis and Ct value as the vertical axis. Calculate the log value of the RNA concentration of the sample from the standard curve based on the Ct value of the sample to be tested, and then calculate its concentration.

4.2. If this kit is used for qualitative testing and only determines positive or negative, the negative control Ct must be greater than 35. The positive control must have fluorescence logarithmic growth, typical amplification curve, and Ct value should be less than 33. For the test sample, if its Ct is ≥ 35, it is negative; if it is ≤ 33, it is positive. If it is between 33-35, repeat once. If the Ct value of the repeated experiment is ≥ 35, it is negative; if it is less than 35, it is positive. The melting curve must also be considered. If the melting Tm value differs from the Tm value of the target amplified fragment by ≥ 2 ℃, it is non-specific amplification and not true positive amplification.