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Rabies Virus Probe qRT-PCR Kit

Product Number:DTK182

Shipping and Storage

Low temperature transportation, stored at -20 ℃, with a shelf life of 12 months.

Component

Component	50T
Probe based qRT PCR buffer	500μL
Probe based qRT PCR enzyme mixture	100μL
Fluorescent PCR specific template diluent	1mL
Rabies virus universal probe method qRT PCR primer probe mixture	150μL
Rabies virus universal probe qRT PCR positive control (1×10E8 copy/μL)	50μL

Note: Self provided reagents: sample RNA, ultrapure water.

Description

Rabies virus (RV) belongs to the family Rhabdoviridae and the genus Rabies virus. It is an RNA virus and is the pathogen that causes rabies. Rabies virus can cause rabies, which is a zoonotic infectious disease that can cause serious damage to human health. Therefore, the rapid and accurate identification of rabies virus plays an important role in the prevention and quarantine of the disease. This product is a universal kit developed specifically for detecting rabies virus based on probe based fluorescence quantitative RT-PCR technology.

Features

1. Ready to use, users only need to provide sample RNA templates.
2. Primers and probes have been optimized for high analytical sensitivity.
3. Provide a positive control to distinguish false negative samples.
4. High specificity, primers are designed based on the highly conserved region of rabies virus and will not cross react with RNA of other viruses.
5. It can be used for both qualitative and quantitative testing. When used for quantitative detection, the linear range should be at least 5 orders of magnitude.
6. This product is sufficient for 50 probe based fluorescence quantitative RT-PCR reactions in a 20μL system.
7. This product can only be used for scientific research.

Protocol

1. Dilute the standard curve sample (using 6 10 fold dilutions of 10E2-10E7 copies/μL as an example).

Due to the high concentration of the standard substance, the following dilution operations must be carried out in a separate area and must not contaminate the sample or other components of this kit. To increase product stability and avoid the spread of infectious pathogens, this product does not provide live samples as positive controls, only non infectious DNA fragments are provided as positive controls.

1.1. Mark 6 centrifuge tubes, namely 7, 6, 5, 4, 3, and 2.

1.2. Add 45μL of fluorescent RT-PCR template dilution solution using a core gun tip, preferably with a core gun tip.

1.3. Add 5μL of 1×10E8 copy/μL positive control (provided by the reagent kit) to tube 7, shake thoroughly for 1 minute, and obtain 1×10E7 copy/μL standard curve sample. Put it on ice for later use.

1.4. Change the gun head and add 5μL of 1×10E7 copy/μL positive control (obtained from the previous dilution) to tube 6. Shake thoroughly for 1 minute to obtain a standard curve sample of 1×10E6 copy/μL. Put it on ice for later use.

1.5. Change the gun head and add 5μL of 1×10E6 copy/μL positive control (obtained from the previous dilution) 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.

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

2. Preparation of Sample RNA

2.1. If there are N samples, it is best to set N+2 extractions, with the additional being PC (positive control for sample preparation) and NC (negative control for sample preparation). You can take 10μL of 1000 fold dilution of the positive control and add a certain amount of water to make the total volume consistent with the specified volume of the sample to be extracted, and use it as PC.

Additionally, use water as NC.

2.2. Purification of RNA samples using self selected methods, this kit is compatible with most RNA extraction kits on the market.

3. Probe qPCR reaction (20μL system, conducted in the sample preparation room)

3.1. If quantitative analysis is performed and only one replicate is performed, label N+9 RT-PCR tubes, including N+2 for the N+2 samples obtained in the previous step, 1 for RT-PCR negative control (using water as a template), and 6 for the standard curve. If qualitative analysis is performed and only one repetition is made, label N+4 RT-PCR tubes, of which N+2 are used for the N+2 samples obtained in the previous step, 1 is used for RT-PCR negative control (using water as a template), and 1 is used for RT-PCR positive control (using the positive control dilution of tube 4 as a template). Below, only quantitative analysis will be used as an example to describe the operational steps.

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 should be added after all tubes are covered and stored):

Ingredients/per tube	N+2 sample tubes	RT-PCR negative control	Standard curve sample tube (2-7 tubes)
Probe based qRT PCR buffer	10μL	10μL	10μL
Probe based qRT PCR enzyme mixture	2μL	2μL	2μL
Rabies virus universal probe method qRT PCR primer probe mixture	3μL	3μL	3μL
N+2 RNA samples to be tested	5μL	-	-
Self provided ultrapure water	-	5μL	-
Step1.6: Standard Curve Sample Dilution Solution (2-7)	-	-	5μL(Sample 2 to tube 2, sample 3 to tube 3...)

3.3. After covering the machine, perform RT-PCR according to the following parameters:

Step	Temperature	Time
Reverse transcription	50℃	20 min
Pre denaturation	95℃	10 min
RT-PCR reaction (45 cycles)	95℃	15sec
	60℃	60sec(Collecting fluorescence signals from FAM channels)

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 test sample, 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 ≥ 40. The positive control must have fluorescence logarithmic growth, typical amplification curve, and Ct value should be ≤ 38. For the test sample, if its Ct is ≥ 40, it is negative; if it is ≤ 38, it is positive. If it is between 38-40, repeat once. If the Ct value of the repeated experiment is ≥ 40, it is negative; if it is < 40, it is positive.