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Rat SDHA Probe-Based Quantitative RT-PCR Kit

Product Number: DTK548

Shipping and Storage

Low temperature transportation, stored at -20℃, with a shelf life of one year. Positive controls need to be placed separately and should not contaminate other reagents.

Component

Component	Specification
2×OneStep Probe Mix	600μL
OneStep Probe Enzyme Mix	60μL
DEPC-H_2O	1mL
Rat SDHA RT-qPCR Primer Mix	60μL
Rat SDHA RT-qPCR probe	30μL
Rat SDHA RT-qPCR positive control (1 × 10E8 copy/μL)	50μL

Description

This kit can be used to detect the SDHA gene in rats. Succinate dehydrogenase flavon (SDHA), as the only active group in the SDH structure, is a key factor in electron transfer and respiratory function of coupled mitochondria.

Application

This product is a rat SDHA detection kit developed based on the principle of probe based fluorescence quantitative PCR. It has the following characteristics:

1. Ready to use, users only need to provide RNA templates.
2. Provide a positive control to distinguish false negative samples.
3. Fast response and high sensitivity.
4. This product is sufficient for 50 fluorescent quantitative RT-PCR reactions using a 20μL probe system.
5. This product can only be used for scientific research.

Specimen collection

Sample RNA, ultrapure water.

Protocol

1. RNA extraction (sample preparation area)

Purification of RNA samples using self selected methods, this kit is compatible with most RNA extraction kits on the market. We recommend using our company's RNA extraction kit to extract samples.

2. Dilute standard curve sample (sample preparation area)

Due to the high concentration of positive control, the following dilution operations must be performed in a separate area to avoid contaminating the sample or other components of this kit.

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

2.2. Add 45μL of DEPC-H_2O separately using a core gun head (preferably using a core gun head, the same below).

2.3. 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.

2.4. Change the gun head and add 5μL of 1 × 10E7 copy/μL positive control (diluted in the previous step) 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.

2.5. 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.

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

If no standard curve is required, dilute the positive control to 1 × 10E5 copies/μL.

3. Reagent Preparation (Reagent Preparation Area)

If there are N samples to be tested, prepare N+2 qPCR tubes (N samples to be tested+1 negative control+6 positive control), and add the following components to each PCR tube:

Ingredients/per tube	N pieces Sample tube to be tested	qPCR negative control tube	qPCR positive control
2×OneStep Probe Mix	10μL	10μL	10μL
OneStep Probe Enzyme Mix	1μL	1μL	1μL
Rat SDHA RT-qPCR Primer Mix	1μL	1μL	1μL
Rat SDHA RT-qPCR probe	0.5μL	0.5μL	0.5μL
DEPC-H_2O	5.5μL	5.5μL	5.5μL

Transfer to the sample preparation area.

4. Add Template (Template Add Area)

Add 2μL of template to qPCR tubes in the following order: negative control (DEPC-H_2O), test sample template, and rat GAPDH RT-qPCR positive control. Centrifuge for 30 seconds and immediately perform amplification reaction.

5. Amplification reaction (amplification and product analysis area)

Place the PCR tube in the corresponding position of the sample slot of the PCR amplification instrument for amplification. The amplification procedure is as follows:

Process	Temperature	Time
Reverse transcription	50℃	15min
Pre denaturation	95℃	3min
qPCR reaction (40 cycles)	95℃	15sec
	60℃	20 sec (collecting fluorescence signal from FAM channel)

6. Result analysis

6.1. If creating a standard curve, plot the 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 determine its concentration.

6.2. If no standard curve has been created, the results shall be judged according to the following criteria:

Positive control result: Ct value<30, with significant exponential growth, showing a typical S-shaped curve.

Negative control result: Ct value>38 or no Ct value, no significant exponential growth period or plateau period.

Sample testing results: Ct value<35, with a significant exponential increase, indicating the detection of the virus in the sample, and the result is positive; A Ct value greater than 38 or no Ct value indicates that the virus was not detected in the sample, and the result is negative; If the Ct value is within the range of 35-38, the sample should be retested. If the Ct value of the repeated experiment is still within the range of 35-38 and there is a significant exponential increase, it is judged as positive. Otherwise, it is judged as negative.