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Human CALC Gene Expression Detection Kit

Product Number: DTK598

Shipping and Storage

Shipping condition: Frozen. Storage: -20℃ up to one-year. Positive control needs to be placed separately, should not contaminate other reagents.

Component

Component	50T
5 ×OneStep Multiplex Probe Mix	280μL
OneStep Multiplex Probe Enzyme Mix	55μL
DEPC-H_2O	1mL
Fluorescent template diluent	1mL
CALCA Gene qPCR primer-probe mix (with Internal Control)	400μL
CALCA Gene qPCR Negative Control (with Internal Control)	100μL
CALCA Gene qPCR positive control (with Internal Control) (1.00E+08 copies/μL)	100μL

Note: The positive control contains both human CALCA and human GAPDH (internal control) target gene fragments, whereas the negative control contains only the human GAPDH target gene fragment.

Description

The kit can be used to detect CALCA Gene. The human CALCA gene (full designation: calcitonin related polypeptide alpha) is a functionally significant gene that gives rise to multiple bioactive peptide hormones via tissue-specific alternative splicing. The principal protein products encoded by this gene include calcitonin, calcitonin gene-related peptide (CGRP), and procalcitonin (PCT). This product is a test for CALCA Gene developed based on the principle of probe based fluorescence quantitative PCR.

Features

1. Ready to use, users only need to provide RNA template.
2. Contains positive control.
3. High specificity, primers are designed based on highly conserved regions of ALV RNA sequences.
4. Applicable to both qualitative or quantitative tests.
5. Sufficient for 50 reactions in a 20μL system.
6. For Research Use Only. Not for use in diagnostic procedures.

Protocol

1. RNA extraction (sample preparation area)

Extract and purify sample RNA first. This kit is compatible with almost all the commercial nucleic acid extraction kits.

2. Dilution of standard curve sample (sample preparation area)

（Due to the high concentration of the positive control, the following dilution operations must be carried out in an independent area to avoid contaminating the samples or other components of the kit.)

2.1. Label 6 centrifuge tubes as 7, 6, 5, 4, 3, and 2.

2.2. Add 45μL fluorescent template diluent individually into the above 6 tubes using filtered pipette tips. (The filtered pipette tips are strongly suggested used in the following procedures.) 3).Add 5μL 1.00E+08 copies/μL Positive control to tube NO.7, vortex for 1 minute, the 1.00E+07 copies/μL of standard curve sample is obtained. Place on ice for later use.

2.3. Change pipette tips, add 5μL 1.00E+07 copies/μL of positive control obtained in Step 3 to tube NO.6, vortex for 1 minute,

the 1.00E+06 copies/μL of standard curve sample is obtained. Place on ice for later use.

2.4. Change pipette tips, add 5μL 1.00E+06 copies/μL of positive control obtained in Step 4 to tube NO.5, vortex for 1 minute, the 1.00E+05 copies/μL of standard curve sample is obtained. Place on ice for later use.

2.5. Repeat the above procedures until a standard curve sample with 6 dilutions is obtained. Place on ice for later use.

If no standard curve is required and only qualitative detection is performed, dilute the positive control to 1.00E+05 copies/μL.

3. Reagent preparation (reagent preparation area)

Prepare sufficient qPCR tubes (test samples，negative control，positive controls). Add the following components to each qPCR tube.

Component	N sample tubes to be tested	qPCR negative control	qPCR positive control
5 ×OneStep Multiplex Probe Mix	5μL each	5μL	5μL
OneStep Multiplex Probe Enzyme Mix	1μL each	1μL	1μL
CALCA Gene RT-qPCR primer-probe mix (with Internal Control)	14μL each	14μL	14μL

4. Add Template (Template Handling Area)

Add 5μL templates to each qPCR tube, in the following order: negative control (DEPC-H_2O), the tested samples and the positive controls. Centrifuge for 30 seconds and immediately perform amplification reaction.

5. Amplification reaction (amplification and product analysis area)

Place the qPCR tubes in the corresponding positions of the sample slot of the qPCR instrument for amplification. The amplification program is as follows:

Step	Temperature	Time
Reverse transcription	50℃	20min
Pre denaturation	95℃	3min
qPCR reaction 45 cycles	95℃	15sec
	60℃	60sec
Signal channel	FAM/HEX	

6. Result Analysis

6.1. Quality Control: The results are considered invalid if any of the following conditions are not met:

Negative Control: No FAM fluorescence signal detected, HEX fluorescence signal detected with a Ct value ≤ 30.0.

Positive Control (1.00E+05 copies/μL): Both FAM and HEX fluorescence signals detected with Ct values ≤ 30.0.

6.2. If a standard curve is prepared: Plot the log value of the Positive Control concentrations on the X-axis against the Ct values on the Y-axis to generate the standard curve. Calculate the log value of the sample RNA concentration from the standard curve using the sample's Ct value, and then determine the concentration.

6.3. If a standard curve is not prepared: Interpret results according to the following criteria:

Sample Results:

Positive: FAM channel Ct value ≤ 38, AND HEX channel Ct value ≤ 30, with a clear exponential growth curve. The sample contains CALCA Gene.

Negative: FAM channel Ct value ≥ 40 or no Ct value, AND HEX channel Ct value ≤ 30. The sample does not contain CALCA Gene.

Indeterminate/Retest: FAM channel Ct value between 38 and 40, AND HEX channel Ct value ≤ 30. The sample should be retested. If the repeat test also yields a Ct value between 38-40 with a clear exponential growth curve, it is considered positive; otherwise, it is negative.

Invalid Sample: HEX channel Ct value > 30.0 or no amplification curve. Re-sampling and re-testing are required.