Instructions for Use of Human Residual Total RNA Detection Kit (RT-PCR)

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG- HR001

Introduction

Human total residual RNA detection kit is used to quantitatively detect the total residual human RNA of various biological products and assist in nucleic acid quality control of biological products.

This kit adopts the principle of the RT-PCR fluorescent probe, combining reverse transcription PCR technology and fluorescent probe method, to realize one-step quantitative detection. Specific primers and probes are designed using human RNA as a template to determine residual RNA content in the test sample by absolute quantification.

Specification

100 Reactions

Main components

Table 1: Kit components

Components	Vial	Volume
One Step RT-qPCR buffer(2x)	1	1ml
One Step Enzyme Mix	1	100μl
Human RNA Primer& Probe Mix	1	370μl
Human RNA Quantitative Standards	1	25μl
RNA IPC Primer&Probe Mix	1	370μl
ROX High (for background calibration in certain instruments; optional based on customer requirements).	1	50μl
ROX Low (for background calibration in certain instruments; optional based on customer requirements).	1	50μl
RNA Diluent	2	1ml

* Please select appropriate ROX for corresponding model.

Product Storage and Shelf Life

The shelf life is 18 months when stored at -20℃ and below.

Applicable models

Including but not limited to real-time quantitative fluorescence PCR instruments such as ABI7500, BioRad CFX96, Bioer FQD-96A and Roche Light Cycler 480.

When using with different instrument models, please select the appropriate reference stain ROX.

Instruments	ROX reference stain
Applied Biosystems® 5700, 7000, 7300, 7700, 7900HT, StepOne™, and StepOnePlus™	ROX High
Applied Biosystems® 7500, ViiA™ 7, QuantStudio™ 12K Flex, Agilent Mx3000P™, Mx3005P™, and Mx4000™	ROX Low
Rotor-Gene™, DNA Engine Opticon™, Opticon™ 2, Chromo 4™ Real-Time Detector, Mastercycler® ep realplex, Smart Cycler®, Roche LightCycle®480, Roche LightCycler®Nano, Bio-Rad CFX96, and IIlumina Eco™	No ROX

Consumables and equipment required

Please prepare the following consumables and equipment before the experiment:

- 1.5 mL or 2 mL sterile low-attachment centrifuge tubes
- 96-well qPCR plate or 8-strip PCR tubes compatible with the PCR system
- Sterile low-attachment pipette tips with cartridge, 1000 µL, 200 µL, and 10 µL specifications
- Centrifuge
- Shaker
- Pipettes of various specifications (such as 1000 µL, 200 µL, 10 µL, and 2.5 µL).
- Fluorescent quantitative PCR system

Procedures

1. Dilution of Human RNA Standards

Dilute human RNA standard (concentration: 2 ng/µL) by gradient with the RNA diluent provided in the kit at concentrations of 200 pg/µL, 20 pg/µL, 2 pg/µL, 200 fg/µL, 20 fg/µL, and 2 fg/µL. The detailed procedures are as follows:

1.1 Place the human RNA standards and RNA dilutions in the kit on ice, allow to completely thaw, mix well by gentle shaking, and centrifuge briefly to settle the liquid to the bottom of the tube.

1.2 Take 6 clean 1.5-mL centrifuge tubes and label as ST0, ST1, ST2, ST3, ST4 and ST5, respectively.

1.3 Dilute the human RNA quantitative reference by 10 folds with RNA diluent in the ST0 tube to obtain ST0. Shake to mix well and quickly centrifuge briefly for 10 s.

1.4 Add 45 µL of RNA diluent into ST0, ST1, ST2, ST3, ST4 and ST5 tubes, respectively.

1.5 Perform 5 dilutions according to Table 2, ensure thorough mixing after each dilution, and then perform the next gradient dilution.

Table 2. Dilution of *E. coli* RNA standard

Standard No.	Dilution volume	Concentration (fg/µL)
STD0	5 µl ST + 45 µl RNase-Free H2O	200
STD1	5 µl ST0 + 45 µl RNase-Free H2O	20
STD2	5 µl ST1 + 45 µl RNase-Free H2O	2
STD3	5 µl ST2+ 45 µl RNase-Free H2O	0.2
STD4	5 µl ST3 + 45 µl RNase-Free H2O	0.02
STD5	5 µl ST4+ 45 µl RNase-Free H2O	0.002

Test sample pretreatment: Test samples should be treated with DNase I before testing, so as to eliminate the influence caused by gDNA. The amount required and digestion conditions of DNase I should be optimized based on the actual sample conditions.

2. Preparation and addition of qRT-PCR reaction solution

2.1 Take out each reagent from the freezer and place them on ice.

2.2 Calculate the required number of reaction wells based on the standard curve and the number of samples to be tested. Generally, 3 replicate wells are required for each sample. That is, the number of reaction wells = (standard curve of 5 concentration gradients + 1 no-template control (NTC) + number of test sample) × 3.

2.3 The total amount of qRT-PCR reaction mixture required for this time is calculated based on the number of reaction wells: qRT-qPCR reaction mixture = (number of reaction wells + 2) × 15 μL (including loss in 2 wells).

2.4 After each reagent has been thawed thoroughly on ice, prepare the reaction Mix as shown in Table 3.

2.5 Thaw each of the above reagents on ice, mix well, and load as shown in Table 4:

Table 3 RT-qPCR Mix Preparation

Components	Reaction in single-well
One Step RT-qPCR buffer(2x)	10 μl
One Step Enzyme Mix	1 μl
Human RNA Primer& Probe Mix	3.6 μl
ROX	0.4 μl
Total volume	15 μl

Table 4 Example of Loading in Each Reaction Well of Standard Curve, NTC and Sample

Standard curve	15 μl RT-qPCR MIX + 5 μl ST1/ST2/ST3/ST4/ST5
NTC	15 μl RT-qPCR MIX + 5 μl RNase Free H2O
Test sample	15 μL of RT-qPCR MIX + 5 μL of test sample

* Select suitable ROX for corresponding model; if the model is suitable for no ROX, add an equal volume of deionized water (it is required to add deionized water without nucleic acid and nuclease contamination).

The total liquid volume in each well is 20 μL after loading.

3. Preparation and addition of IPC reaction solution

3.1 IPC tests for no-template control (IPC-NTC) and each test sample (IPC-S) in each experiment are required as shown in Table 5 and Table 6.

Table 5 IPC RT-qPCR MIX Preparation

Components	Reaction in single-well
One Step RT-qPCR buffer(2x)	10 μl
One Step Enzyme Mix	1 μl
RNA IPC Primer&Probe Mix	3.6 μl
ROX	0.4 μl
Total volume	15 μl

Table 6. Examples of loading to each reaction well

IPC-NTC	15 μl IPC RT-qPCR MIX + 5 μl RNase Free H_2O
IPC-S1	15 μL IPC RT-qPCR MIX + 5 μL test sample S1
IPC-S2	15 μL IPC RT-qPCR MIX + 5 μL test sample S2
IPC-S3	15 μL IPC RT-qPCR MIX + 5 μL test sample S3

The total volume of each well after the completion of loading is 20 μL.

3.2 Design the plate layout as per the following table

Table 7. Example of a 96-well plate layout

ST1	ST1	ST1										A
ST2	ST2	ST2										B
ST3	ST3	ST3							IPC-S1	IPC-S1	IPC-S1	C
ST4	ST4	ST4							IPC-S2	IPC-S2	IPC-S2	D
ST5	ST5	ST5							IPC-S3	IPC-S3	IPC-S3	E
									S1	S1	S1	F
				NTC	NTC	NTC			S2	S2	S2	G
				IPC-NTC	IPC-NTC	IPC-NTC			S3	S3	S3	H
1	2	3	4	5	6	7	8	9	10	11	12	

This example represents tests for a DNA standard curve of samples at five concentration gradients, a NTC, and three test samples. IPC tests for IPC-NTC and IPC test samples (IPC-S1, IPC-S2, IPC-S3). prepare wells in triplicate. During actual testing, samples in the 96-well plate may be loaded based on the actual number of test samples, as per the plate layout in Table 7.

3.3 Seal the 96-well plate with an optical plate sealer, gently shake to mix well, perform rapid centrifugation with a centrifuge dedicated for 96-well plate to let all liquid gather at tube bottom, then place the plate in the qPCR system.

4. Run

ABI 7500 qPCR instrument (software V2.0.6) is used as an example.

4.1 Create a blank new procedure and select Absolute Quantitative Test Template and Taqman Probe Method.

4.2 Create a new detection probe named as Human RNA, select reporter fluorophore as FAM and quench fluorophore as none; create a new detection probe named as RNAIPC, select reporter fluorophore as VIC and quench fluorophore as none; and detection reference fluorescence is ROX.

4.3 Set up a three-step reaction program: 50 ℃ for 15 min; 45 cycles of 95 ℃ for 30 s; 95 ℃ for 10 s, 60 ℃ for 40 s; and a reaction volume of 20 μL.

4.4 Run the PCR program.

Result analysis

1. In the "Amplification Plot" of Results, the user may initially check whether the amplification curve is of normal shape. Usually, the system will automatically set the threshold and baseline. Multiple thresholds may be generated in case of different target settings, leading to inaccurate result analysis. In such case, please manually set the threshold line, which must be within the exponential amplification region, for example, the threshold is usually set to 0.15. Select "Auto Baseline", and click "Analyze" to view the adjusted results.

2. In Standard Curve in Results, the slope, intercept, R^2 of the standard curve can be read.

3. In the Report in Results, the Mean Quantity column reads the test values in pg/μL for the no-template control (NTC) and the test sample. For NTC, the Ct value should be greater than that of ST5, with a difference being no less than 4, and the detection value should be lower than 2 fg/μL.

4. Analyze the Ct value of IPC. Normally, the Ct-IPC value of the test sample should be consistent with the that of NTC, or within in the range of Ct-IPC value of NTC ± 1. If the Ct-IPC value of the test sample is significantly higher than that of NTC, it indicates that there may be significant inhibition in the test sample.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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