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GAPDH（Human）Real Time RT-PCR Kit User Manual

LT022310RQ50

For use with ABI Prism®7000/7300/7500/7900/Step One Plus; iCycler iQ™4/iQ™5; Smart Cycler II;Bio-Rad CFX 96;Rotor Gene™6000; Mx3000P/3005P;MJ-Option2/ Chromo4; LightCycler®480 instrument

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Σ 50 −20 °C CE

1. Intended Use

GAPDH real time RT-PCR Kit is used for the detection of GAPDH gene in human samples (blood, tissue, and etc.) by using real time PCR systems.

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is one of the most commonly used housekeeping genes used in comparisons of gene expression data. GAPDH as a housekeeping gene is typically a constitutive gene that is transcribed at a relatively constant level. It is widely used in PCR system as an internal control .Therefore, this kit can be used with other RT-PCR kits which are used for detection of other target genes.

GAPDH real time RT-PCR kit contains a specific ready-to-use system for the detection of the GAPDH using RT-PCR (Reverse Transcription Polymerase Chain Reaction) in the real-time PCR system. The master contains Super Mix for the specific amplification of GAPDH. The reaction is done in one step real time RT-PCR. The first step is a reverse transcription (RT), during which the GAPDH is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by means of PCR (polymerase chain reaction). Fluorescence is emitted and measured by the real time systems optical unit during the PCR. The detection of amplified GAPDH fragment is performed in fluorimeter **channel FAM** with the fluorescent quencher BHQ1.

4. Kit Contents

Ref.	Type of reagent	Presentation	50rxns
1	GAPDH Super Mix	1 vial, 480µl	x2
2	RT-PCR Enzyme Mix	1 vial, 28µl	x2
3	Molecular Grade Water	1 vial, 400µl	x2
4	GAPDH Positive Control(1×10^5~1×10^6 copies/ml)	1 vial, 30µl	x2
5	GAPDH QS1(1×10^8copies/ml)	1 vial, 20µl	x2
6	GAPDH QS2(1×10^7copies/ml)	1 vial, 20µl	x2
7	GAPDH QS3(1×10^6copies/ml)	1 vial, 20µl	x2
8	GAPDH QS4(1×10^5copies/ml)	1 vial, 20µl	x2

Analysis sensitivity: 200 copies/ml LOQ: 1×10^3～1×10^8copies/ml

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors .If you use the RNA extraction kits recommended, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Super Mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological cabinet
- Vortex mixer
- Cryo-container
- Sterile filter tips for micro pipets
- Disposable gloves, powderless
- Refrigerator and Freezer
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)
- Real time PCR system
- Real time PCR reaction tubes/plates
- Pipets (0.5µl – 1000µl)
- Sterile microtubes
- Biohazard waste container
- Tube racks

7. ⚠Warnings and Precaution

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Quickly prepare the reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.

8. Sample Collection, Storage and transport

- Collected samples in sterile tubes.
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

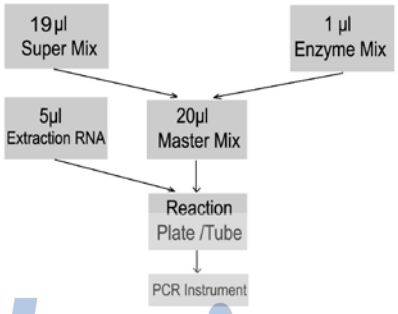
9. Procedure

9.1 RNA-Extraction

RNA extraction is recommended the same as target gene RNA extraction.
e.g. Calibration for BCR-ABL fusion gene, please do RNA extraction from Leukocytes according to instructions of Leukemia BCR-ABL Fusion Gene Kit.

9.2 RT-PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



1) The volumes of Super Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
2) Pipet **20µl** Master Mix with micropipets of sterile filter tips to each of the real time PCR reaction plate/tubes. Separately add **5µl** RNA sample template, positive，QS1~QS4 and negative controls to different plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

45°C for 20min	1cycle
95°C for 5min	1cycle
95°C for 15sec, 58°C for 30sec, 72℃×45sec (Fluorescence measured at 58°C)	45cycles

Selection of fluorescence channels	
FAM	GAPDH gene

5) ⚠ If you use ABI Prism® system, please choose **"none"** as **passive reference** and **quencher**.

10. Threshold setting: just above the maximum level of molecular grade water.

11. Calibration for quantitative detection: Input each concentration of standard controls at the end of run, and a standard curve will be automatically formed.

12. Quality control: Negative control , QS1~QS4 and positive control must be performed correctly, otherwise the sample results is invalid.

Channel / Control	Ct value
	Channel FAM
Molecular Grade Water	UNDET
Positive Control(qualitative assay)	1×10^5~1×10^6 copies/ml
QS（quantitative detection）	Correlation coefficient of QS curve≤－0.98

13. Data Analysis and Interpretation : The following results are possible:

	Ct value	Result Analysis
1	UNDET	Below the detection limit or negative
2	＜200 copies/ml	positive; its concentration lower than 200 copies/ml
3	200~1×10^3copies/ml	positive; the quantitative value for recommendation only
4	1×10^3~1×10^8copies/ml	positive; the quantitative value is valid
5	＞1×10^8copies/ml	1) positive; but the quantitative value for recommendation only 2)Re-test the sample after dilute the sample by several times, making the quantitative value within 10^3~10^8 copies/ml

13.1 Concentration of GAPDH gene in blood sample (copies/ml)

$$\frac{V1（ml）\times C1（copies/ml）}{V2（ml）}$$

13.2 Concentration of GAPDH gene in each leukocyte (copies/cell)

$$\frac{V1（ml）\times C1（copies/ml）}{V2（ml）\times C2（cell/ml）}$$

V1=Elution volume； V2=Sample Volume；
C1=concentration of quantitative value from the PCR software
C2=concentration of leukocyte in blood sample

13.3. Calibration

$$\text{Target gene mRNA levels (\%)} = \frac{\text{Concentration of target gene}\times100\%}{\text{Concentration of GAPDH}}$$

For further questions or problems, please contact our technical support at **trade@liferiver.com.cn**