Human Telomerase Reverse Transcriptase (hTERT) mRNA One Step RT-PCR Quantification kit

（48T）

Store at －15 to －25℃

Cat#:BSB04M1

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4. PCR protocol

Vial	Program	Cycle Program Data	Value	
1.	**Reverse transcription**	Cycles	1	
		Analysis Mode	None	
		Temperature Targets	***Segment 1***	***Segment 2***
		Target temperature	90℃	61℃
		Incubation time (h:min:s)	0:00:30	0:20:00
		Analysis mode	None	None
2	**Denaturation**	Cycles	1	
		Analysis Mode	None	
		Temperature Targets	***Segment 1***	
		Target temperature	95℃	
		Incubation time (h:min:s)	0:02:00	
		Analysis mode	None	
3	**PCR amplification**	Cycles	40	
		Analysis Mode	Quantification	
		Temperature Targets	***Segment 1***	***Segment 2***
		Target temperature	95℃	60℃
		Incubation time (h:min:s)	0:00:15	0:01:00
		Analysis mode	None	Single(FAM)

5. Results and Data Analysis

Perform data analysis, as described in the Real Time PCR Detection Instrument Operator's Manual.Analysis method Use of the Fit points method. This renders the method independent from user-born influences.

6. Quality control

1) The C_t value of the Negative control must be ∞.
2) The Correlation of standard curve must be ≤-0.98.

detect telomerase in various sample materials.The Human Telomerase Reverse Transcriptase (hTERT) mRNA One Step RT-PCR Quantification kit is intended as research tool facilitating the generation of data to proof these hypothesis's.

This Quantification Kit is adapted for many kinds of Real Time PCR Detection Instrument., specifically adapted for Line-Gene I&II Real-time PCR Detection System. hTERT -encoding mRNA is reverse transcribed and a fragment of the generated cDNA is amplified with specific primers in a one-step RT-PCR reaction.The amplicon is detected by using a specific Taqman-MGB Probes.This Taqman-MGB Probes is labeled at the 5'-end with FAM report dye , and the 3'-end by NFQ(Non Fluorescent Quencher)-MGB.

Kit contents and Storage

Function	Name	contents	Storage
Sample treat kit	Biozol	100ml×1 bottle	2～8℃
			Not pack in this Kit
RT-PCR Kit	RT-PCR MIX	0.5ml×2	-15～-25℃
	Mn2+	110ul×1	-15～-25℃
	hTERT probe Mix	200ul×1	-15～-25℃
	DEPC H_2O	1.0ml×2	-15～-25℃
hTERT Standard	Standard 1（1～9×10^7copy/ml）	40ul×1	-15～-25℃

Once the kit was opened, store the kit components as described in the before table.Thaw the solutions on ice and, for maximal recovery of contents, quick-spin vials in a microcentrifuge above opening. After mixing gently, the solutions should be stored in aliquots. Repeated freezing and thawing must be avoided.

Additional required reagents and equipment

Chloroform, isopropanol(-20℃ Cooled), 75%ethanol(-20℃ Cooled), Nuclease-free aerosol-preventive pipette tips

• Sterile reaction (Eppendorf) cups for preparing dilutions

• standard benchtop microcentrifuge containing a rotor for 2.0 ml reaction tubes. 0.2 ml realtime PCR tube

Sample material

Purified RNA or messenger RNA (mRNA) from

- cell cultures
- scientific biopsy material
- whole blood
- other biological samples

hTERT Standard

Use DEPC H_2O dilute Standard 1（1～9×10^7copies/ml）for 10 times to Standard 2（1～9×10^6copy/ml）; then use DEPC H_2O dilute Standard 2（1～9×10^6copies/ml）for 10 times to Standard 3（1～9×10^5copy/ml）; finally use DEPC H_2O dilute Standard 3（1～9×10^5copies/ml）for 10 times to Standard 4（1～9×10^4copy/ml）.

Protocol

1. Sample Preparations

For preparation of total RNA or mRNA from cell cultures or any biological material, refer to the Biozol user's manual. For most applications, e.g. total RNA from cell cultures, optimal results are obtained when using 100–200 ng total RNA per reaction. For special applications the amounts may vary and should be adapted. The flexibility of the assay allows also the use of cDNA, as starting material.

2. Preparation of solutions

Each individual run on the Real-time PCR Detection System consists of:

- 4 hTERT standards for quantification of hTERT mRNA expression levels,
- 1 H_2O control reactions for controlling the complete RT-PCR process,
- And a variable number of RNA samples to be analyzed for relative hTERT expression.

3. Preparation of the master mixes

Define the experimental protocol before preparing the solutions. E.g. as indicated above,when analyzing 8 samples per run 5 + 8 = 13 reactions with hTERT Master Mix.

Depending on the number of samples to be analyzed, calculate the number of reactions needed plus one additional reaction for the hTERT Master Mix Proceed as described below for a 40 ul standard reaction when preparing the master mixes and the reaction mixtures.

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Preparation of the master mixes:
Note: The volumes indicated below are based on a single 40ul standard reaction.Prepare hTERT master mixes by multiplying the amount in the ”Volume”column by the number of reactions to be cycled, plus one additional reaction. It is recommended preparing a mixture containing RT-PCR MIX, Mn2+, hTERT probe Mix and DEPC H2O, in a 1.5 ml reaction tube first, before dividing the mixture in separate vials and adding the Detection Mixes.
The volumes per tube are mentioned below:
<table>
<tr><td>Component</td><td>RT-PCR MIX</td><td>Mn^{2+}</td><td>hTERT probe Mix</td><td>DEPC H2O</td></tr>
<tr><td>Volume</td><td>20 ul</td><td>2 ul</td><td>4 ul</td><td>10 ul</td></tr>
</table></td></tr>
<tr><td>2</td><td>● Mix gently.
● Pipette 36 ul master mix into the corresponding realtime PCR tube.
● Add 4 ul of the corresponding RNA template (hTERT standards 1 to 4, H_2O controls).</td></tr>
<tr><td>3</td><td>● Cap each tube
● Put the tube into a standard benchtop microcentrifuge.
● Centrifuge at 700 × g for 5s (3000 rpm in a standard benchtop microcentrifuge).
Note: Place the centrifuge adapters in a balanced arrangement within the centrifuge.</td></tr>
<tr><td>4</td><td>● Place the PCR tube in the Real Time PCR Detection Instrument.</td></tr>
<tr><td>5</td><td>● Cycle the samples as described in follows</td></tr>
<tr><td>6</td><td>● In the Edit Sample Screen define the site containing the hTERT standards 1-4 as standard, and enter the respective concentrations, provided with the kit in the lot-specific information . Make sure, all other samples are defined as unknown.
● Note: More detailed information on options of the Edit Sample Screen is given in the Real Time PCR Detection Instrument Operator's Manual.</td></tr>
</table>

Introduction

Telomeres are specialized DNA/protein structures located at the end of eukaryotic chromosomes. Telomeres play an essential role in the stable maintenance of the eukaryotic chromosome within a cell by serving as specific binding sites for structural proteins.These proteins cap the ends of linear chromosomes, thus preventing nucleolytic degradation, end-to-end fusion, irregular recombination, and other events that are normally lethal to a cell. Additionally, telomeres are required to maintain nuclear architecture and interact with other proteins to repress the expression of adjacent genes (1).

A progressive shortening of the chromosome ends with each replication cycle was proposed to result from DNA polymerase´s inability to replicate linear DNA to its very ends (so-called “end-replication problem”) (2). This phenomenon, which has been demonstrated in vitro and in vivo, seems to be linked to the limited proliferative capacity of normal somatic cells (“mitotic clock”). Assuming correctness of a.m. hypothesis, germline cells, stem cells, and tumor cells, which are able to proliferate with a prolonged or even infinite life span, must possess a mechanism for maintaining telomere length (3).The maintenance of stable telomere length in replicating cells is associated with the activation of telomerase. For human telomerase, the name “human Telomerase Reverse Transcriptase” (hTERT) is also used. Telomerase is a ribonucleoprotein, consisting of the actual telomerase enzyme and a “Telomerase-associated RNA” (e.g. hTR, for human telomerase-associated RNA). The enzyme's activity compensates for the loss of telomeric DNA by adding repeat sequences to the chromosome ends. As a result,telomerase acts as a reverse transcriptase, that uses part of its intrinsic RNA component as a template for telomeric repeat synthesis (4,5). The genes encoding its RNA subunit and the catalytic protein subunit have been cloned from a variety of species, including humans (6,7,8). Both subunits are essential for restoring telomerase activity in vitro, and introduction of these genes into normal human cells can extend the life span of these otherwise mortal cells (9,10).

Numerous data on telomerase expression demonstrate the presence of telomerase activity in the vast majority of different types of cancer - as well as immortalized cells -but fail to detect telomerase in most normal tissues (11). Using the highly sensitive PCR-based TRAP method (12), telomerase activity has been detected in most neoplastic lesions and appears to be necessary for the sustained proliferation of most advanced cancers. Examples include breast cancer, neuroblastoma, and cervical cancers where a correlation between telomerase expression and the stage of the disease has been demonstrated (11). Therefore the presence of telomerase enzyme activity in tumors might lead to a useful marker for diagnosis and prognosis of cancer in the future. Determination of expression levels of telomerase-encoding mRNA presents an alternative approach to

Use note

1. This kit is for research use only.
2. Before you begin,you should read this user's manual carefully.
3. Prepare appropriate aliquots of the kit solutions and keep them separate from other reagents in the laboratory.
4. The use of nuclease-free labware (e.g. pipettes, pipette tips, reactions vials)as well as
5. Wearing gloves when performing the assay.
6. To avoid cross-contamination of samples and reagents use fresh aerosol-preventive pipette tips for all pipetting steps .
7. To avoid carry-over contamination, transfer the required solutions for one experiment into a fresh tube instead of directly pipetting from stock solutions.
8. Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.
9. To minimize risk of carry-over contamination, it is worthwhile to physically separate the workplaces for RNA preparation.