Quantitative Telomerase Detection Kit
(QTD Kit)

Catalog No. MT3010, MT3011, MT3012

US Biomax

For Research Use Only.
Not for use in diagnostic procedures

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1. Introduction:

Background

Telomerase is an enzyme that synthesized telomeres on chromosome ends. Telomerase activity is specifically associated with immortal cells including cancer cells. A highly sensitive *in vitro* assay known as the Telomeric Repeat Amplification Protocol has been developed for detecting telomerase activity in cells and tissues. In the assay, viable or freshly frozen cells/tissues (with enzymatically active telomerase) are lysed and the telomerase activity in the cell extract is determined through its ability to synthesize telomeric repeats onto an oligonucleotide substrate *in vitro* upon the addition of the appropriate buffer conditions and dNTPs. Telomerase from the cell extract adds telomeric repeats onto a substrate oligonucleotide and the resultant extended product are subsequently amplified by the polymerase chain reaction (PCR). The PCR products are then visualized using highly sensitive DNA fluorochromes SYBR Green. Direct detection of PCR product is monitored by measuring the increase in fluorescence caused by the binding of SYBR Green dye to double-strand DNA.

Product Overview

Quantitative Telomerase Detection Kit Description:

Quantitative Telomerase Detection Kit (QTD Kit) is specifically designed for Real-time PCR detecting telomerase activity.

The QTD kit is a convenient premix which contains all the components except template sample (cell or tissue extract) and water necessary to perform real-time PCR or PAGE analysis using SYBR green I dye. Direct detection of PCR product is monitored by measuring the increase in fluorescence caused by the binding of SYBR Green dye to double-strand DNA.

Quantitative Telomerase Detection Kit Content:

Catalog Number	MT3010	MT3011	MT3012
Reaction contains	50	100	200
2 x QTD Premix	0.65 ml	1.25 ml	2 x 1.25 ml
1× Lysis Buffer*	1.8 ml	2 x 1.8 ml	3 x 1.8 ml
PCR Qualified Water	1.8 ml	2 x 1.8 ml	2 x 1.8 ml
Control Template TSR (0.5 amol/µl)	20 µl	20 µl	20 µl

* Add protease inhibitor cocktail before use as needed.

Storage and Stability:

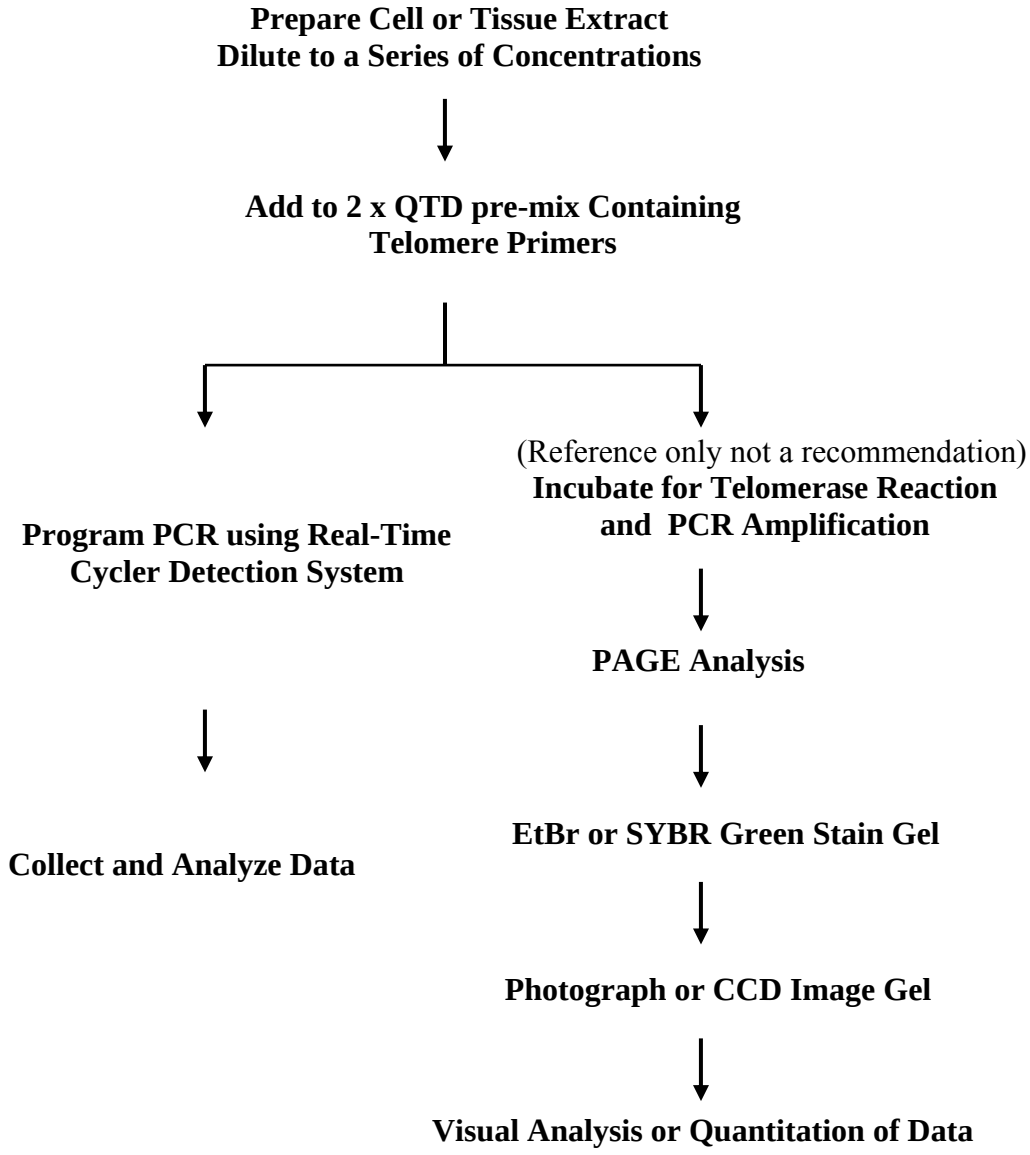
Quantitative Telomerase Detection Kit can be stored at -20°C up to 12 months without showing any reduction in performance.

Warnings and Precautions

1. Since the Quantitative Telomerase Detection Kit detects the activity of telemerase, a RNase sensitive ribonucleoprotein, and not merely the presence of the RNA or protein components of telemerase, the assay requires enzymatically active cell or tissue samples. Furthermore, due to the sensitivity of the QTD Real-Time PCR kit assay, which can detect telomerase activity in a very small number of cells, a special laboratory setup and significant precautions are required to prevent PCR carry over contamination and RNase contamination.
2. Quantitative Telomerase Detection Kit is designed, developed, and sold for research purpose only. It is not to be used for human diagnostic or drug purposes. When working with chemicals, always wear a suitable lab coat, disposable gloves.

2. QTD Real-Time PCR Assay Procedure

Experimental Scheme of QTD Real-Time PCR Assay



Telomerase Assay Protocols

Extract Preparation

1. Pellet cells or tissue, wash once with PBS, repellet, and carefully remove PBS, the cells or tissue pellet can be stored at –80°C. At this condition, Telomerase in frozen cells or tissue is stable for at least a year. When thawed for extraction the cells or tissue should be resuspended immediately in 1× Lysis Buffer* (add protease inhibitor cocktail as needed).
2. Resuspend the cell pellet in 200 µl of 1× Lysis Buffer /10^5-10^6 cells. Use 200 µl 1× Lysis Buffer /40-100 mg of tissue.
3. Incubate the suspension on ice for 30 minutes.
4. Spin the sample in a microcentrifuge at 12,000x g for 30 minutes at 4°C.
5. Transfer 160 µl of the supernatant into a fresh tube and determine the protein concentration.
6. Aliquot and quick-freeze the remaining extract on dry ice/ethanol, and store at -80°C

Assay Controls

a. Heat inactivation control:

Telomerase is a heat-sensitive enzyme. As a negative control, every sample extract to be evaluated should also be tested for heat sensitivity. Thus, analysis of each sample consists of two assays: one with a test extract and one with a heat-treated test extract. Heat treat extract by incubating at 85°C for 10 minutes prior to the telomerase activity assay.

b. TSR control template standard curve:

TSR is an oligonucleotide with a sequence similar to Telomere primers. Perform Quantitative Real-Time PCR using dilutions of TSR to generate a standard curve. This standard curve allows the calculation of the amount of template with telomeric repeat created by telomerase in a given extract.

The stock TSR (control template) concentration provided within the kit is 0.5 amoles/µl. Prepare 1:5 serial dilutions of the stock concentration with Lysis Buffer to obtain TSR concentration of 0.1 amoles/µl, 0.02 amoles/µl, 0.004 amoles/µl, 0.0008 amoles/µl, 0.00016 amoles/µl. Perform the telomerase detection standard assay using 1 µl of each TSR dilution including the 0.5 amoles/µl stock concentration. The TSR dilutions can be stored at 4°C for at least 2 weeks.

Telomerase Activity Assay using QTD real-Time PCR

Real-time PCR reactions

1. Prepare a master mix by mix the reagents outlined below except for the extract. Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the cycler.

   Reaction components for each assay:

2 x QTD Premix	12.5 µl
Cell or Tissue Extract	1.0 µl
PCR Qualified Water	11.5 µl
Total Volume	25.0 µl

2. Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes or thin-wall PCR plates.
3. Add 1 µl of test extract, heat-inactivated extracts or template controls to the individual PCR tubes containing the master mix.
4. Program Real-Time PCR Detection System according to the program outlined below:

Program of real-time cycler;

Steps	Time	Temperature	Comments
Telomerase Reaction	20 min	25°C	Telomere templates were formed by adding 6-base-repeats to primer with the activity of telomerase
PCR Initial Activation Step	10 min	95°C	HotActivitedTag DNA Polymerase is activated by this heating step
3 -step cycling:			
Denaturation	30s	95°C	
Annealing	30 s	60°C	
Extention	30 s	72°C	
Cycle number	35-40 cycles		Cycle number depends on the amount of template DNA.

5. Place the PCR tubes in the thermal cycler and start the cycling program.
6. Real-Time detection. The PCR Quantification screen is displayed during the PCR run and presents data as they are being collected in real time. Collect the

threshould cycle or C_T value after cycles finished. The threshould cycle is the cycle at which a statistically significant increase in Δ Rn is first detected.

7. Threshold is defined as the average standard deviation of Rn for the early cycles, multiplied by an adjustable factor. On the graph shown below, the threshold cycle occurs when the increased signal associated with an exponential growth of PCR product was begin to be detected.

Data Analysis

Generation of the TSR template control standard curves

The following is a quantitative result of positive control template TSR using QTD Real-Time PCR assay. A standard curve was generated using the reading of the threshold (C_T) of Real-Time PCR.

Real-Time Threshold cycles of TSR template

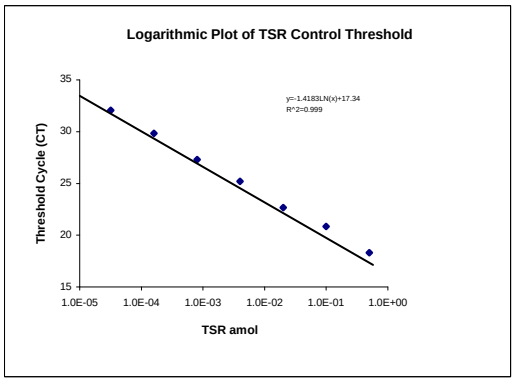
Dilutions	TSR concentration		Threshold cycles (C_T)
	(amol /μl)	molecules/reaction	
S1	0.5	300000	18.3
S2	0.1	60000	20.8
S3	0.02	12000	22.6
S4	0.004	2400	25.2
S5	0.0008	480	27.3
S6	0.00016	96	29.8
S7	0.000032	20	32.0
S8	0.0000064	4	32.5
Blank	-	-	33.0

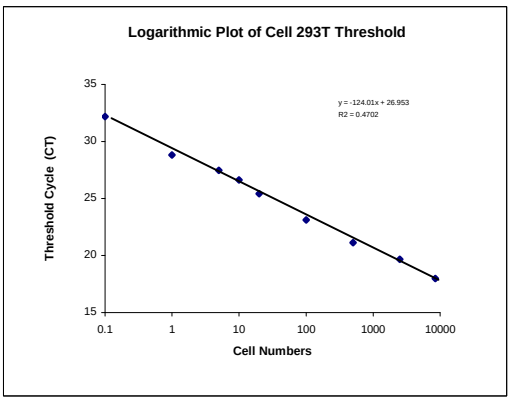
Comparison of Telomerase Activity in Various Extract

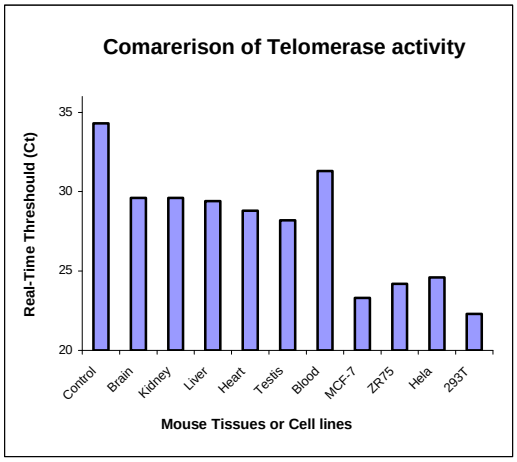
Real-Time Threshold (C_T) of cell extract from 293T

Simple Dilutions	Cell Numbers Per reaction	Threshold cycles (C_T)
A	8500	18.0
B	2500	19.7
C	500	21.1
D	100	23.1
E	20	25.4
F	10	26.6
G	5	27.5
H	1	28.8

Blank	--	32.6 or N/A







3. Troubleshooting guide

The following are the troubleshooting guide designed to provide a convenient optimization procedure for real-time PCR using the Real-Time QTD kit.

Comments and Suggestions

A. No significant increase in fluorescence signals observed.

1. PCR annealing time too short
   Use the recommended annealing time 30-45 seconds.
2. Extension time too short.

Use the extension time specified in the protocols.

3. HotActivitedTaq DNA polymerase not activated
   Ensure that the cycling program included the HotActivitedTaq DNA polymerase activation step (15 minutes at 95°C).
4. PCR annealing temperature too high
   Decrease annealing temperature in 2°C steps.
5. Insufficient telomerase in cell extract.
   Perform the cell or tissue extraction on ice, always keeps minimum preparation time.
6. Insufficient telomerase reaction time.
   Always use the reaction time specified in the protocol. Incubation time is 20 minutes.
7. No detection activated
   Check the flurescence detection was activated in the cycling program and ensure the flurescence takes place during the extension step of the PCR program.

B. No linearity in ratio of C_T value/crossing point to log of the template amount

Template amount too high
Do not exceed maximum recommended amount

C. All reaction samples and negative controls show a positive threshold.

PCR carry-over contamination.
Use fresh aliquots of every component of the assay contents.
Be sure to use clean PCR tubes and PCR tube racks.
Separate the preparative area from the PCR amplification and detection area

D. A high fluorescence signal is observed with heat-treated extracts.

Extracts are not heat sensitive or insufficient heat inactivation
Check the temperature of the heatblock or water bath used for heat inactivation of the extract.
PCR carry-over contamination
Refer to problem C.

4. Reference

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