Quantitative Telomerase Detection Kit (QTD Kit)

Catalog No. MT3010, MT3011, MT3012

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:

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

* 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 telomerase, a RNase sensitive ribonucleoprotein, and not merely the presence of the RNA or protein components of telomerase, 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

Prepare Cell or Tissue Extract
Dilute to a Series of Concentrations

Add to 2 x QTD pre-mix Containing Telomere Primers

(Reference only not a recommendation)
Incubate for Telomerase Reaction and PCR Amplification

Program PCR using Real-Time Cycler Detection System

PAGE Analysis

Collect and Analyze Data

EtBr or SYBR Green Stain Gel

Photograph or CCD Image Gel

Visual Analysis or Quantitation of Data
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 /10⁵-10⁶ 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 mixing 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:

<table>
<thead>
<tr>
<th>2 x QTD Premix</th>
<th>12.5 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell or Tissue Extract</td>
<td>1.0 µl</td>
</tr>
<tr>
<td>PCR Qualified Water</td>
<td>11.5 µl</td>
</tr>
<tr>
<td>Total Volume</td>
<td>25.0 µl</td>
</tr>
</tbody>
</table>

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:

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

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
threshold cycle or $C_T$ value after cycles finished. The threshold cycle is the cycle at which a statistically significant increase in $\Delta R_n$ is first detected.

7. Threshold is defined as the average standard deviation of $R_n$ 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**

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

**Comparison of Telomerase Activity in Various Extract**

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

<table>
<thead>
<tr>
<th>Dilutions</th>
<th>Cell Numbers Per reaction</th>
<th>Threshold cycles ($C_T$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8500</td>
<td>18.0</td>
</tr>
<tr>
<td>B</td>
<td>2500</td>
<td>19.7</td>
</tr>
<tr>
<td>C</td>
<td>500</td>
<td>21.1</td>
</tr>
<tr>
<td>D</td>
<td>100</td>
<td>23.1</td>
</tr>
<tr>
<td>E</td>
<td>20</td>
<td>25.4</td>
</tr>
<tr>
<td>F</td>
<td>10</td>
<td>26.6</td>
</tr>
<tr>
<td>G</td>
<td>5</td>
<td>27.5</td>
</tr>
<tr>
<td>H</td>
<td>1</td>
<td>28.8</td>
</tr>
</tbody>
</table>
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 fluorescence detection was activated in the cycling program and ensure the fluorescence takes place during the extension step of the PCR program.

B. No linearity in ratio of CT 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


