

## Telomerase PCR ELISA

### For fast and sensitive detection of telomerase activity

Boehringer Mannheim's Telomerase PCR ELISA is designed for the highly sensitive qualitative detection of telomerase activity in cell extracts from cell cultures and other biological samples.

Telomeres are specialized DNA-protein structures found at the end of eukaryotic chromosomes. Telomeric DNA is characterized by an array of tandemly repeated, G-rich DNA sequences that are highly conserved during evolution. Telomeres are believed to play an important role by protecting the genomic DNA from degradation and deleterious recombination events, such as end-to-end fusion, rearrangements, chromosomal translocations, and chromosomal loss. As DNA polymerase  $\alpha$  is unable to replicate the extreme ends of linear DNA, every replication cycle leads to progressive shortening of the telomeric ends in normal somatic cells. This phenomenon has been demonstrated *in vitro* and *in vivo*, and it appears to be linked to the limited proliferative capacity of normal somatic cells of higher eukaryotes. It is likely that this activity plays a role in events related to cellular senescence ("mitotic clock," see Greider and Blackburn, *Scientific American*, February 1996, pages 80–85).

In contrast to somatic cells, germline cells are perpetual, and are required to preserve the genome's full information for future generations of an organism. To achieve this, they must circumvent the fatal effects associated with telomere shortening. This is accomplished by adding new telomeric repeat sequences to the ends of germline chromosomes. Telomerase, a ribonucleoprotein, catalyzes the addition of TTAGGG repeats to the ends of vertebrate chromosomes, using a complementary sequence of its intrinsic RNA component as a template. Telomerase activity was also shown to be expressed in most cell lines and tumors (see Kim *et al.* (1994) *Science* **226**: 2011–2015). Escaping from the proliferative limitations of cellular senescence, telomerase reactivation might be a prerequisite for the development of malignant tumor cells from somatic cells.

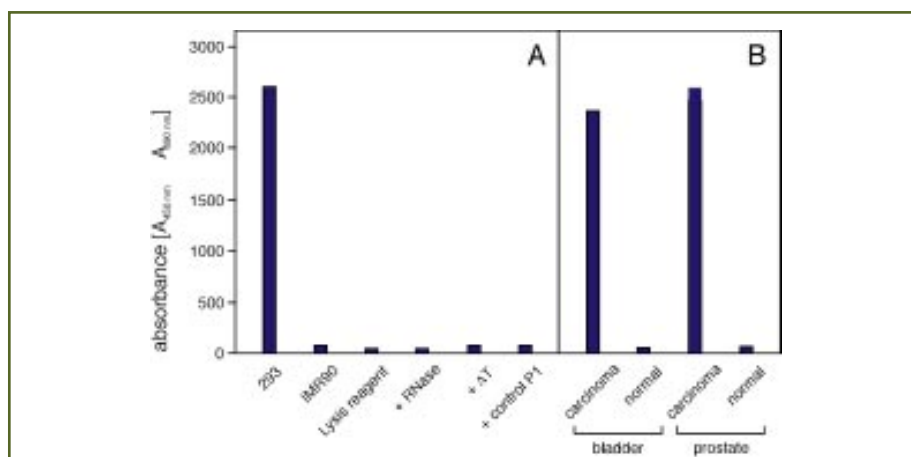
The conventional primer-extension-based assay for detecting telomerase activity requires large amounts of cells or tissue, and it only detects telomerase with

limited sensitivity. Those disadvantages have been overcome by the Telomeric Repeat Amplification Protocol (TRAP; see Kim *et al.*, 1994), in which the telomerase reaction product is amplified by PCR. Until now, the TRAP assay provided full sensitivity only when used with a radioactive label, and it required visualization of results by autoradiography after gel electrophoresis, which is both hazardous and time consuming.

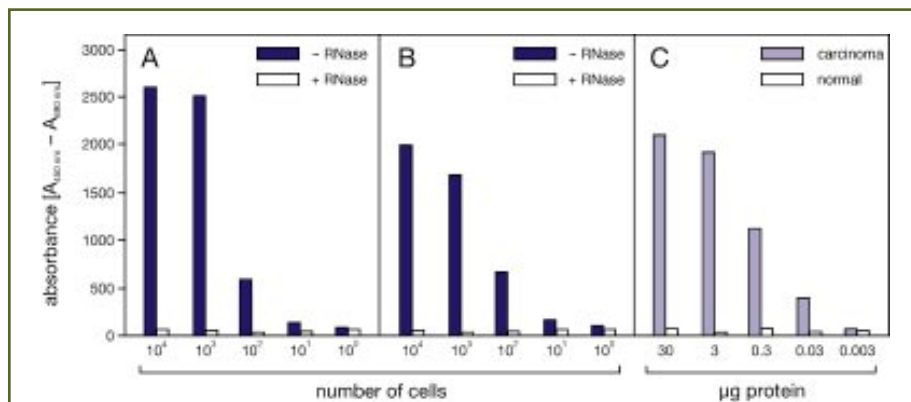
The Telomerase PCR ELISA Kit provides a way to perform a highly sensitive photometric enzyme immunoassay for the detection of telomerase activity, using non-radioactive ELISA techniques. The assay can be separated into the following steps:

### Elongation/amplification

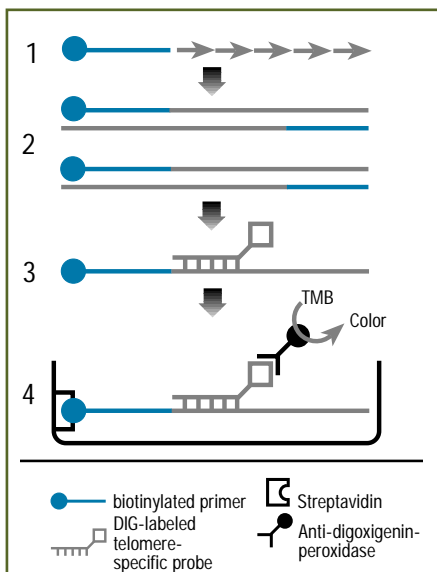
Telomerase adds telomeric repeats (TTAGGG) to the 3' end of the biotin-labeled synthetic P1-TS-primer. Then,



**Figure 7** Specific detection of telomerase activity in cell lines and tissue samples. (A) Human, telomerase-positive embryonic kidney cell line 293 (293) and human, telomerase-negative lung fibroblasts (IMR90) were analyzed in the Telomerase PCR ELISA. For negative controls, one of the following was used: Lysis reagent without extract (Lysis reagent), 239 cells treated with RNase (+ RNase), or 239 cells that were heat-treated (+ DT). All of these controls gave negative results. Control P1 is a synthetic oligonucleotide that is not accepted by telomerase as a substrate. Assays were performed as described in section 5 with amounts of extracts equivalent to  $1 \times 10^3$  cells. (B) Telomerase activity in normal and primary tumor tissue derived from biopsy. A prostate carcinoma and a bladder carcinoma were compared to normal prostate and bladder tissue (normal) respectively. Assays were performed as described in section 5, using 20  $\mu$ g total protein.



**Figure 8** Sensitivity of the Telomerase PCR ELISA. (A) An extract of telomerase-positive 293 cells was serially diluted with Lysis reagent, and the indicated cell equivalents were analyzed as described in section 5. Results indicate extracts that were treated with RNase (+ RNase) or not treated with RNase (- RNase). (B) HEK293 cells were serially diluted in culture medium before lysis, then treated with Lysis reagent as described in section 5. The indicated number of cells were analyzed in the Telomerase PCR ELISA. Assays were performed as described in section 5, and results indicate samples that were treated with RNase (+ RNase) versus samples without RNase treatments (- RNase). (C) Telomerase activity in serially diluted extracts obtained from a bladder carcinoma tumor and from normal bladder tissue. The indicated amounts of tissue material were assayed for telomerase activity.



**Figure 9** Detection of telomerase activity with the Telomerase PCR ELISA.

Step 1. Telomerase, if present, adds multiple 6-nucleotide telomeric repeats to a biotinylated synthetic primer.

Step 2. The telomerase reaction product is amplified by PCR, using a biotinylated primer.

Step 3. After denaturation, the PCR product hybridizes to a digoxigenin-labeled probe specific for the telomeric repeat.

Step 4. The DNA hybrid binds to a streptavidin-coated microtiter plate, and anti-digoxigenin-peroxidase and TMB substrate generate a colored product measurable with a microplate reader.

**Note:** If desired, the TRAP reaction product from Step 2 can also be detected by the traditional gel electrophoresis method.

these elongation products are amplified by PCR with the primers P1-TS and P2, generating PCR products with the telomerase-specific 6-nucleotide increments. As opposed to other TRAP assay formats and home brew reagents, the Telomerase PCR ELISA contains all compounds required for the telomerase reaction and PCR in a ready-to-use reaction buffer. An added benefit is that this format combines both reactions in one-step/one-tube-reaction. Additionally, optimized primer sequences eliminate the need for hot start PCR or separation of the primers by a wax barrier and avoids amplification artifacts, such as primer dimers.

### Detection by ELISA

An aliquot of the PCR product is denatured and hybridized to a digoxigenin- (DIG-) labeled, telomeric repeat-specific detection probe. The resulting product is immobilized via the biotin-labeled primer to a streptavidin-coated microtiter plate. The detection probe and the hybridization conditions have been optimized to obtain the highest specificity and sensitivity (Figures 7,8). The immobilized PCR product is then detected with an antibody against digoxigenin that is conjugated to peroxidase (anti-DIG-POD). Finally, the probe is visualized by virtue of peroxidase metabolizing TMB to form a colored reaction product.

### Visualizing the telomerase-specific 6 bp ladder (optional):

The ELISA utilizes a biotinylated primer for immobilization within the ELISA microtiter plate. Using it together with the Biotin Luminescence Detection Kit, this biotin label can also serve as a means of detection. If the typical, telomerase-mediated 6-nucleotide ladder is desired, the fragments can be separated by PAGE, blotted onto a positively charged membrane, and detected appropriately (e.g., with the Biotin Luminescent Detection Kit, Cat. No. 1 811 592).

### Advantages of the Telomerase PCR ELISA

- The Telomerase PCR ELISA shows a sensitivity comparable to the radioisotopic TRAP assay (Figure 10) but without the risk of using radioisotopes.
- The premade TRAP reaction mix minimizes the risk of assay failure resulting from contamination, and it avoids the need for time-consuming preparation of solutions.
- The ready-to-go mixtures allow performance of telomerase-mediated primer elongation and PCR amplification in one tube (one-step reaction).

- The Telomerase PCR ELISA delivers results very quickly (within 6–8 h) and allows simultaneous analysis of up to 96 TRAP reactions.
- It enables different ways of detecting the TRAP reaction products. The products can be detected in an ELISA format with the reagents provided in the kit, or alternatively, the reaction products can be visualized after gel electrophoresis and subsequent blotting and detection.

Product	Cat. No.	Pack Size
Telomerase PCR ELISA* <sup>§</sup>	1 854 666	1 kit (96 tests)

Also Available	Cat. No.	Pack Size
RNase, DNase-free	1 119 915	500 mg
Nylon Membrane, positively charged	1 209 272	10 sheets, 20 x 30 cm
	1 209 299	20 sheets, 10 x 15 cm
	1 417 240	1 roll, 0.3 x 3 m
Biotin Luminescence Detection Kit	1 811 592	50 blots a 10 x 10 cm
Aerosol-resistant pipette tips <sup>†</sup>	1 667 068	960 tips (0.1–10 µl)
	1 667 076	1000 tips (0.5–10 µl)
	1 667 084	960 tips (2–20 µl)
	1 667 173	960 tips (40–20 µl)
Thin-walled PCR Tubes <sup>†</sup>	1 667 041	1000 tubes, 200 µl
	1 667 050	1000 tubes, 500 µl

<sup>†</sup> These products are sold under licensing arrangements with Roche Molecular Systems and The Perkin-Elmer Corporation.

\* Purchase of these products is accompanied by a limited license to use them in the Polymerase Chain Reaction (PCR) process in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased, i.e., an authorized thermal cycler.

<sup>§</sup> Licensed from Geron Corporation.