For life science research only.
Not for use in diagnostic procedures.

*Telo**TAGGG*** Telomerase PCR ELISA

Version: 10

Content Version: December 2020

Photometric enzyme immunoassay for the detection of telomerase activity, utilizing the Telomeric Repeat Amplification Protocol (TRAP)

Cat. No. 11 854 666 910 1 kit
96 reactions

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	black	*Telo*TAGGG Telomerase PCR ELISA, Lysis reagent	▪ Ready-to-use solution. ▪ For the preparation of cell extracts from cell cultures and tissue samples.	1 bottle, 22 ml
2	yellow	*Telo*TAGGG Telomerase PCR ELISA, Reaction mixture, 2x conc.	▪ Ready-to-use solution. ▪ Tris-buffer, containing Telomerase substrate, primers, nucleotides, and Taq polymerase. ▪ Provides one-step telomerase-mediated primer elongation and PCR amplification. ▪ Includes the biotin-labeled P1-TS primer and P2 primer.	1 bottle, 3 ml
3	violet	*Telo*TAGGG Telomerase PCR ELISA, Denaturation reagent	▪ Ready-to-use solution. ▪ Contains <0.5% sodium hydroxide.	2 bottles, 1.5 ml each
4	white	*Telo*TAGGG Telomerase PCR ELISA, Hybridization buffer	▪ Ready-to-use solution. ▪ Contains a DIG-labeled detection probe complementary to telomeric repeat sequences.	1 bottle, 25 ml
5	green	*Telo*TAGGG Telomerase PCR ELISA, Washing buffer, 10x conc.	For washing steps.	1 bottle, 50 ml
6	red	*Telo*TAGGG Telomerase PCR ELISA, Anti-DIG-POD	▪ Polyclonal antibody from sheep. ▪ Lyophilized and stabilized.	1 bottle, 120 mU
7	red	*Telo*TAGGG Telomerase PCR ELISA, Conjugate dilution buffer	Ready-to-use solution.	1 bottle, 12 ml
8	black	*Telo*TAGGG Telomerase PCR ELISA, TMB substrate solution	▪ Ready-to-use solution. ▪ Contains the POD substrate 3,3′,5,5′-tetramethylbenzidine.	1 bottle, 12 ml
9	blue	*Telo*TAGGG Telomerase PCR ELISA, Stop reagent	▪ Ready-to-use solution. ▪ Contains <5% sulfuric acid.	1 bottle, 12 ml
10	colorless	*Telo*TAGGG Telomerase PCR ELISA, Positive control	▪ Cell extract prepared from immortalized telomerase-expressing human kidney cells (293 cells). ▪ Lyophilized	1 bottle, 20 µl
11	foil bag	*Telo*TAGGG Telomerase PCR ELISA, Microplate	▪ Precoated with streptavidin and post-coated with blocking reagent. ▪ Shrink-wrapped with a desiccant capsule (12 × 8 wells).	1 strip frame, 12 modules of 8 wells each
12	–	*Telo*TAGGG Telomerase PCR ELISA, Self-adhesive Plate Cover Foil	Prevents evaporation. ⚠ ***Cover the Microplate modules with the Cover Foils during each incubation step.***	3 foils

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	black	Lysis reagent	Store in aliquots at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	yellow	Reaction mixture, 2x conc.	Thaw Reaction mixture on ice and dispense solution into appropriate aliquots. ⓘ *25 µl are required for one reaction.* Store aliquots at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
3	violet	Denaturation reagent	Store in aliquots at +2 to +8°C.
4	white	Hybridization buffer	Thaw solution on ice and dispense solution into appropriate aliquots. ⓘ *225 µl are required for one reaction.* Store aliquots at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
5	green	Washing buffer, 10x conc.	Store in aliquots at +2 to +8°C.
6	red	Anti-DIG-POD	Store at −15 to −25°C.
7	red	Conjugate dilution buffer	Store in aliquots at +2 to +8°C for 6 months.
8	black	TMB substrate solution	
9	blue	Stop reagent	Store in aliquots at +2 to +8°C.
10	colorless	Positive control	Store in aliquots at −80°C.
11	foil bag	Microplate	Store dessicated at +2 to +8°C. Stable for approximately 12 months once the bag has been opened.
12	–	Self-adhesive Plate Cover Foil	Store at +2 to +8°C.

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Sterile aerosol-preventive pipette tips
- Pipettes with disposable positive-displacement tips
- Sterile reaction tubes for preparing dilutions
- Microplate shaker (preferably +37°C)
- Microplate washer (optional)
- Microcentrifuge
- ELISA reader for microplates with 450 nm filter.
  ⓘ *Use a reference wavelength of approximately 690 nm.*

For preparation of kit working solutions

- Autoclaved, double-distilled water
- DEPC-treated water (optional)

For preparation of negative control

- RNase, DNase-free*

For preparation of extracts from cells

- Neubauer hemocytometer
- Refrigerated centrifuge
- Reaction tubes
- Phosphate-buffered saline (PBS)*

For preparation of extracts from tissue

- Cryostat
- Sterile reaction tubes

For telomeric repeat amplification protocol (TRAP)

- PCR thermal cycler
- Mineral oil
- Tubes for PCR amplification

For nonradioactive detection after transfer to nylon membranes

- 12% nondenaturating acrylamide gel
- Loading dye
- Electrophoresis equipment
- Nylon membranes, positively charged*
- 2% Blocking reagent*
- Streptavidin-alkaline phosphatase (AP) conjugate*
- Chemiluminescence equipment for the visualization of blotted products

The TeloTAGGG Telomerase PCR ELISA is designed for performing hybridization and detection in combination with a plate shaker at 300 rpm. If a shaker is not available, perform hybridization in reaction tubes as described in section, **Protocols**. *The detection steps can be carried out as described in section,* **Protocols**.

1.4. Application

The *Telo*TAGGG Telomerase PCR ELISA is designed for the highly sensitive qualitative detection of telomerase activity in cell extracts from cell cultures and other biological samples. The kit utilizes a biotinylated primer for immobilization within the ELISA microplate. Using it together with a biotin-detection system, this biotin label can also serve as a means of detection; If the typical, 6-nucleotide-ladder, resulting from the TRAP assay is desired, the fragments can be separated by polyacrylamide gel electrophoresis (PAGE), blotted onto a positively charged membrane, and detected appropriately.

1.5. Preparation Time

Assay Time

Step	Approximate Duration [hours]
Extract preparation	1
Elongation and amplification	2.5
Hybridization and photometric detection	2.5
Total hands-on time	1
Total assay time	**7**

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The *Telo*TAGGG Telomerase PCR ELISA can be used with:
- Cell cultures
- Scientific biopsy material
- Other biological research samples.

Control Reactions

Preparation of negative control

Telomerase requires integrity of its internal RNA component as a template for the addition of the telomeric repeat sequences to the telomerase-specific primer. Therefore, preincubation of the cell or tissue extract with RNase, DNase-free* will fully destroy telomerase activity contained in the extract and offers a convenient control of specificity. Alternatively, heat-treatment of the cell extract for 10 minutes at +85°C prior to the TRAP reaction may be used to inactivate telomerase protein for producing negative controls. The following protocol is recommended:

1. Incubate 5 µl of cell extract typically corresponding to 5,000 cell equivalents or 50 µg of protein if extract from tissue samples is used with 1 µl RNase, DNase-free (1 µg/µl) for 20 minutes at +37°C.

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2. An aliquot of 1 to 3 µl of the RNase-treated extract is used for the *Telo*TAGGG Telomerase PCR ELISA.

Incubation of a telomerase-positive extract at +37°C for 20 minutes in the absence of RNase does not affect the telomerase-specific signals. Therefore, any loss of signal is due to specific degradation of the internal RNA component. Exert extreme care to avoid carryover of RNase into samples, resulting in false-negative readings.

With this RNase treatment, the maximum value of absorbance for the negative control should be 0.25 $A_{450\ nm}$ to $A_{690\ nm}$ units. If values are higher, repeat the entire experiment including the TRAP reaction.

General Considerations

Precautions

Determining telomerase activity using the *Telo*TAGGG Telomerase PCR ELISA requires both the addition of telomeric repeats to a primer by the activity of telomerase (contained in the sample), and their subsequent amplification by PCR.
Consequently, extreme caution is imperative to prevent:
- RNase/DNase contamination that might cause degradation of the internal, telomerase-associated RNA template.
- PCR carryover contamination resulting in false-positive signals.

As opposed to other TRAP assay formats, the *Telo*TAGGG Telomerase PCR ELISA, provides all of the compounds required to perform telomerase-mediated primer elongation and the PCR reaction in a ready-to-use master mix. However, to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions:
- Use only double-distilled water that is nuclease-free, for example, DEPC-treated and autoclaved.
- Prepare appropriate aliquots of the kit solutions and keep them separate from other reagents in the laboratory.
- Use autoclaved or heat-sterilized labware, such as pipettes, pipette tips, and reactions vials.
- Wear gloves and a surgical mask during cell extraction and when performing the assay.
- Use fresh aerosol-preventive pipette tips for all pipetting steps to avoid cross-contamination of samples and reagents.
- Physically separate the workplaces for sample preparation, TRAP reaction, and detection of amplicons to minimize the risk of carryover contamination.

Number of tests

Up to 94 samples plus control reactions can be performed, depending on the number of samples analyzed per experiment.

Safety Information

The following reagents used in the assay are toxic or corrosive and must be handled with care:
- Denaturation reagent (Bottle 3)
- TMB substrate solution (Bottle 8)
- Stop reagent (Bottle 9)

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Solution	Content	Reconstitution/Preparation of Working Solution	Storage and Stability	For use in...
5	Washing buffer, 1x conc. (Bottle 5)	▪ Dilute an appropriate volume of Washing buffer, 10x conc. with autoclaved, double-distilled water (1:10) and mix thoroughly. ▪ Approximately 2.5 ml of the diluted Washing buffer is needed for one reaction.	Store at +2 to +8°C for one month.	Washing steps
6a	Anti-DIG-POD, stock solution (Bottle 6)	▪ Quickly spin Bottle 6 before opening. ▪ Reconstitute the lyophilizate in 240 µl autoclaved, double-distilled water. ▪ This results in an antibody conjugate concentration of 0.5 U/ml.	Store at +2 to +8°C for 6 months. ⚠ ***Do not freeze.*** ⚠ ***Do not add sodium azide.***	Preparation of Solution 6b.
6b	Anti-DIG-POD, working solution	▪ Dilute an appropriate amount of the reconstituted Anti-DIG-POD (Solution 6a) with Conjugate dilution buffer (Bottle 7) to a final concentration of 10 mU/ml, for example, 100 µl antibody solution and 4.9 ml of Conjugate dilution buffer.	Prepare immediately before use.	Labeling reaction
10	Positive control (Bottle 10)	▪ Quickly spin Bottle 10 before opening. ▪ Reconstitute lyophilized cell extract on ice with 20 µl autoclaved, double-distilled water (optionally treated with DEPC) and mix thoroughly. ▪ The reconstituted solution has a concentration of approximately 1 × 10^3 cell equivalents per microliter. ▪ Dispense solution into suitable aliquots; 1 to 3 µl will be needed for one reaction. ⚠ ***Keep the extract on ice during pipetting.***	Store at −80°C. ⚠ ***Avoid repeated freezing and thawing.***	Control reactions
11	Microplate	Use only the Microplate modules required for the particular experiment. Close the foil bag containing the remaining modules and the desiccant capsule tightly with adhesive tape. ⓘ *The Microplate modules are ready-to-use and do not need to be rehydrated before use.*	Once the foil bag is opened, store Microplate modules desiccated at +2 to +8°C for approximately 12 months.	ELISA assay

2.2. Protocols

Preparation of extracts from cells

⚠ ***To avoid carryover contamination, transfer the required volumes of Bottles 1, 2, and 10 for one experiment into a fresh tube instead of directly pipetting from stock solutions.***

1. Harvest and count cells using a hemocytometer.

2. Transfer 2 × 105 cells per single reaction into a fresh reaction tube.

3. Pellet cells at 3,000 × g for 10 minutes in a refrigerated centrifuge at +2 to +8°C.

4. Carefully remove supernatant and resuspend the cells in PBS; repeat the centrifugation step.

5. Carefully remove the supernatant.
   – If the TeloTAGGG Telomerase PCR ELISA is not performed immediately after the extract preparation, store the pelleted cells at −80°C until use.

6. Resuspend the pelleted cells in 200 µl Lysis reagent (Bottle 1), pre-cooled on ice by reverse pipetting at least 3 times and incubate on ice for 30 minutes.
   If frozen cell pellets were used for extraction, thaw cell pellets on ice before adding Lysis reagent (Bottle 1).

7. Centrifuge the lysate at 16,000 × g for 20 minutes in a refrigerated centrifuge at +2 to +8°C.

8. Carefully remove the supernatant and transfer to a fresh tube.
   – To ensure that no cellular debris of the pelleted cells is transferred, pipette only 175 µl of the cell extract.

9. Perform the TRAP reaction as described below.
   – If not immediately performing the TRAP reaction, shock freeze the cell extract in aliquots in liquid nitrogen and store the extracts at −80°C.

Preparation of extracts from tissue

Preparing tissue specimens for determining telomerase activity requires careful acquisition and storage of research materials since cross-contamination with tumor cells can result in false-positive signals in normal tissues or negative signals in tumor samples due to improper storage.

Tissue samples should be shock frozen in small pieces in liquid nitrogen, and can be stored at −80°C if not used immediately in the TeloTAGGG Telomerase PCR ELISA. However, we recommend that extracts be stored, which have been prepared as described below, rather than storing tissue specimens.

1. Prepare cryostat sections of 10 to 15 µm thickness from frozen tissue samples.

2. Transfer approximately 50 sections into a sterile reaction tube containing 200 µl ice-cold Lysis reagent (Bottle 1).
   Alternatively, If a microtome is not available, thin slices of frozen tissue specimens may be prepared on sterile disposable petri dishes with surgical disposable knife blades to obtain thin flakes, which are then immediately transferred to homogenization tubes containing 200 µl ice-cold Lysis buffer (Bottle 1). Homogenize on ice with a motorized pestle until uniform consistency.

3. Incubate on ice for 30 minutes.

4. Centrifuge the lysate at 16,000 × g for 20 minutes in a refrigerated centrifuge at +2 to +8°C.
   – A refrigerated benchtop centrifuge using reaction tubes can also be used.

5 Carefully remove the supernatant and transfer to a fresh tube.
– To ensure that no debris of the tissue is transferred, pipette only 175 µl of the tissue extract.

6 Measure the protein concentration by standard methods; shock freeze the tissue extract in aliquots in liquid nitrogen and store the extracts at −80°C.

Telomeric repeat amplification protocol (TRAP reaction)

The cycling program mentioned below has been established for use with the GeneAmp PCR System 9600 Thermal Cycler and the DNA Thermal Cycler 480 (Perkin Elmer).
Depending on the performance characteristics of other licensed thermal cyclers, minor modifications of the protocol might be required. Before starting the amplification process for some thermal cyclers, it may be necessary to overlay the reaction mixture with mineral oil to prevent water condensation at the top of the tube. It has been shown in a number of experiments that overlaying mineral oil on top of the reaction mixture already for the telomerase-mediated primer elongation does not influence the results.

1 For each sample to be tested and the controls, transfer 25 µl Reaction mixture (Bottle 2) into a tube suitable for PCR amplification.

2 Add the following volumes as mentioned in the table.
i Perform all pipetting steps on ice.

Samples/control	Volume
Samples	1 to 3 µl cell extract per tube, corresponding to 1 × 10^3 to 3 × 10^3 cell equivalents, or 1 to 50 µg total protein, see section, **Preparation of extracts from cells**.
Negative controls	1 to 3 µl of the corresponding RNase treated or heat-treated cell extract, corresponding to 1 × 10^3 to 3 × 10^3 cell equivalents, or 1 to 50 µg total protein.
Positive control	1 to 3 µl of the reconstituted solution, corresponding to 1 × 10^3 cell equivalents.

– Add sterile water to a final volume of 50 µl.

3 Transfer tubes to a thermal cycler and perform a combined primer elongation/amplification reaction:

Step	Time	Temperature [°C]	Cycles
Primer elongation	10 – 30 min	25	1
Telomerase inactivation	5 min	94	1
Amplification: Denaturation Annealing Polymerization	 30 sec 30 sec 90 sec	 94 50 72	 1 – 30
Final extension	10	72	1
Hold	–	4	–

Hybridization and ELISA

1. Per sample, transfer 20 µl of Denaturation reagent (Bottle 3) into a suitable reaction tube.
   For a large numbers of samples, use a nuclease-free, uncoated microplate.

2. Add 5 µl of the amplification product and incubate at +15 to +25°C for 10 minutes.

3. Add 225 µl Hybridization buffer (Bottle 4) per tube and mix thoroughly by vortexing briefly.
   The amount of the hybridization mixture allows the detection of the amplicons to be performed in duplicates, if desired.

4. Transfer 100 µl of the mixture per well of the precoated Microplate modules supplied with the kit and cover the wells with the Self-adhesive Cover Foil to prevent evaporation.

5. Incubate the Microplate modules at +37°C on a shaker at 300 rpm for 2 hours.
   If an appropriate microplate shaker is not available, perform the hybridization reaction in a reaction tube, and after shaking for 2 hours at +37°C at 300 rpm, transfer 100 µl of the hybridization mixture per well of the precoated Microplate modules.– Cover the wells with the Self-adhesive Cover Foil and incubate for an additional 1 hour at +37°C.

6. Remove the Hybridization solution completely.– Wash 3 times with 250 µl of Washing buffer (Solution 5) per well for a minimum of 30 seconds each; carefully remove Washing buffer.

7. Add 100 µl Anti-DIG-POD working solution (Solution 6b) per well.– Cover the Microplate modules with a Cover Foil and incubate at +15 to +25°C for 30 minutes while shaking at 300 rpm.

8. Remove the solution completely.– Rinse 5 times with 250 µl of Washing buffer (Solution 5) per well for a minimum of 30 seconds each; carefully remove Washing buffer.

9. Add 100 µl TMB substrate solution (Bottle 8) prewarmed at +15 to +25°C per well.– Cover the wells with a Cover Foil and incubate for color development at +15 to +25°C for 10 to 20 minutes while shaking at 300 rpm.

10. Without removing the reacted substrate, add 100 µl Stop reagent (Bottle 9) per well to stop color development.
    Addition of the Stop reagent causes the reacted POD substrate to change in color from blue to yellow, and is required to achieve maximal sensitivity.

11. Using a Microplate (ELISA) reader, measure the absorbance of the samples at 450 nm, using a reference wavelength of approximately 690 nm within 30 minutes after addition of the Stop reagent.

Nonradioactive detection of telomerase-mediated DNA ladder in extracts of cultured cells after transfer to nylon membranes

The biotin-labeled P1-TS Primer used in the TeloTAGGG Telomerase PCR ELISA enables easy analysis of the amplification products, see section, **Telomeric repeat amplification protocol** by Southern hybridization, if proof for the presence of the typical, telomerase-mediated 6-nucleotide-ladder is desired.

i *During development and external evaluation of the Telomerase PCR ELISA, a strict correlation of positive results in the TeloTAGGG Telomerase PCR ELISA and procedures, producing the typical TRAP ladder was found.*

1. Prepare cell extracts and perform the TRAP reaction as previously described.

2. Mix loading dye containing Bromophenol blue and xylene cyanol with 20 µl of the PCR product.
   – Perform polyacrylamide gel electrophoresis according to standard protocols, using a 12% nondenaturing acrylamide gel.
   – Apply current until Bromophenol blue runs off the gel following standard procedures.
   i *The smallest telomerase product band should be 52 bp.*

3. After electrophoresis, transfer PCR products by vacuum blotting onto a positively charged nylon membrane* using standard techniques.

4. Block the membrane with a solution of 2% Blocking reagent* to prevent nonspecific binding for 30 minutes at +15 to +25°C.

5. Discard Blocking solution and incubate the membrane with a streptavidin-alkaline phosphatase (AP) conjugate*, diluted appropriately.

6. Rinse membrane 2 times in Washing buffer for 15 minutes at +15 to +25°C.

7. After rinsing the membrane, visualize the blotted products by chemiluminescence.

2.3. Parameters

Sensitivity

The *TeloTAGGG* Telomerase PCR ELISA kit provides a way to perform a highly sensitive photometric enzyme immunoassay for the detection of telomerase activity using nonradioactive techniques.

3. Results

Interpretation of results

Absorbance values are reported as the $A_{450\ nm}$ reading against blank (reference wavelength $A_{690\ nm}$).

Negative control

As described in section, **Control Reactions**, an appropriate negative control for checking the specificity of the telomerase reaction, is degrading the telomerase-associated RNA by preincubating the cell extract with DNase-free RNase. Alternatively, heat-treatment may be used to prepare negative controls. With this RNase treatment, the maximum value of absorbance for the negative control should be 0.25 $A_{450\ nm}$ to $A_{690\ nm}$ units. If values are higher, repeat the entire experiment including the TRAP reaction.

Positive control

The absorbance readings obtained with the Positive control (Bottle 10) should be >1.5 $A_{450\ nm}$ to $A_{690\ nm}$ units after 20 minutes substrate reaction when using 1 × 10^3 cell equivalents in the assay. If values are lower, repeat the entire experiment including the TRAP reaction.

Samples

Subtract the mean of the absorbance readings of the negative controls from those of the samples. Samples are regarded as telomerase-positive if the difference in absorbance (ΔA) is >0.2 $A_{450\ nm}$ to $A_{690\ nm}$ units.

Typical results

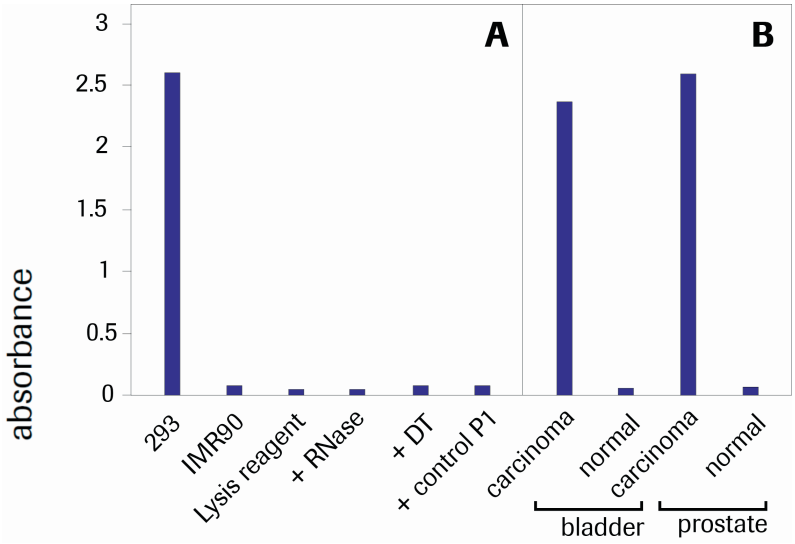

Fig. 1: Specific detection of telomerase activity in cell lines and tissue samples.
(A) Human, telomerase-positive embryonic kidney cell line (293) and human, telomerase-negative lung fibroblasts (IMR90) were analyzed by the *Telo*TAGGG Telomerase PCR ELISA. For negative controls, one of the following was used: Lysis reagent without extract (Lysis reagent), 293 cell extracts treated with RNase (+ RNase), or heat (ΔT). 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 according to the kit protocol with amounts of extracts equivalent to 1 × 10^3 cells.
(B) Telomerase activity was measured in normal and primary tumor tissue derived from biopsy. A prostate carcinoma and a bladder carcinoma were compared to normal prostate and bladder tissue, respectively. Assays were performed according to the kit protocol, using 20 µg total protein.

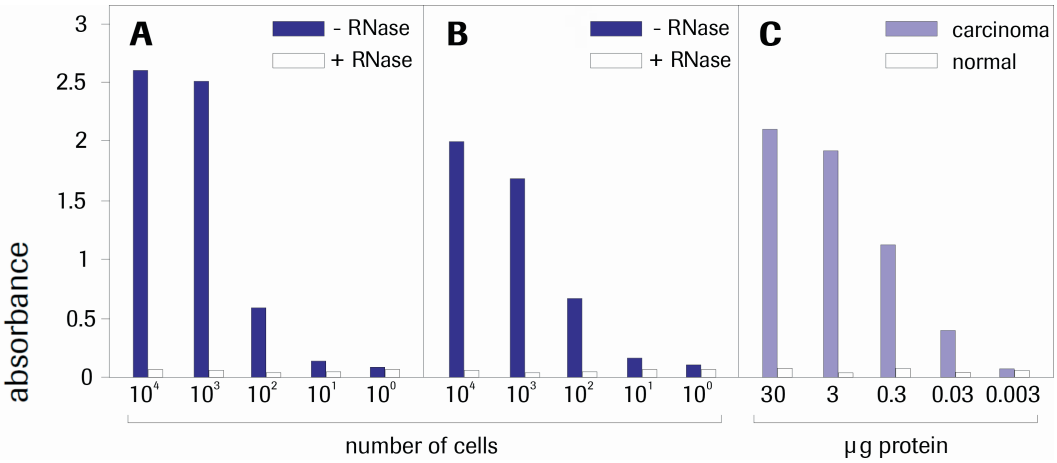

Fig. 2: Sensitivity of the*Telo**TAGGG* Telomerase PCR ELISA.
(A) An extract of telomerase-positive 293 cells was serially diluted with Lysis reagent, and the indicated cell equivalents were analyzed. Results indicate extracts that were treated with RNase (+ RNase) or not treated with RNase (- RNase).
(B) 293 cells were serially diluted in culture medium before lysis, then treated with Lysis reagent. The indicated number of cells were analyzed by the*Telo**TAGGG* Telomerase PCR ELISA. Assays were performed according to the kit protocol, and results indicate samples that were treated with RNase (+ RNase) versus samples without RNase treatments (- RNase).
(C) Telomerase activity in serially diluted extracts were obtained from a bladder carcinoma (carcinoma) and from normal bladder tissue (normal). The indicated amounts of tissue material were assayed for telomerase activity.

4. Troubleshooting

Observation	Possible cause	Recommendation
Negative control signals too high.	Negative control, Lysis reagent, or Reaction mixture may be contaminated with a telomerase-positive cell extract.	Inactivate an aliquot of the negative control by RNase-treatment or heating for 10 minutes at +85°C; repeat the entire experiment including amplification.
	Negative control, Lysis reagent, Reaction mixture, or the other reagents may be contaminated with amplification products from previous experiments.	Prepare new negative control and fresh reagents.
	Washing when performing the detection step was not sufficient.	Increase number of washing steps.
	Incubation with TMB substrate solution was too long.	Stop substrate reaction within 20 minutes of adding the TMB substrate solution.
Positive control signals too low or absent.	Thermal cycling program is not appropriate for the type of thermocycler used.	Optimize cycling conditions.
	Water used for the combined primer elongation/amplification was not free of nucleases.	Use only PCR-grade water* or double-distilled water treated with DEPC and autoclaved for reconstitution of kit components and preparation of buffers.
	Reagents were contaminated with nucleases.	Repeat the entire assay with fresh aliquots of Positive control cell extract and samples.
		Check reagents for the presence of DNase and RNase contaminations.
	Incubation steps were performed without shaking at 300 rpm.	Absolute levels of absorbance mentioned above were obtained when a microplate shaker was used.
	Anti-DIG-POD working solution is inactive.	Use only freshly prepared Anti-DIG-POD working solution.
		Check enzyme activity of the Anti-DIG-POD working solution and prepare fresh working solution.
	Inadequate storage of Positive control templates and/or other kit components.	Check storage conditions, see section, **Storage and Stability**.

5. Additional Information on this Product

5.1. Test Principle

The *TeloTAGGG* Telomerase PCR ELISA enables highly specific amplification of telomerase-mediated elongation products combined with nonradioactive detection following an ELISA protocol (Fig. 3).

1. During elongation, telomerase adds telomeric repeats (TTAGGG) to the 3′ end of the biotin-labeled synthetic P1-TS-primer.

2. Elongation products are amplified by PCR using 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 brews, the TeloTAGGG Telomerase PCR ELISA contains all compounds required for the telomerase reaction and PCR in a ready-to-use reaction buffer for combining both reactions in a one-step/one-tube-reaction. An additional advantage over the conventional assay is the use of optimized primer sequences eliminating the need for hotstart PCR or separation of the primers by a wax barrier, avoiding amplification artifacts, such as primer-dimers.

3. An aliquot of the PCR product is denatured and hybridized to a digoxigenin-(DIG)-labeled, telomeric repeat-specific detection probe.

4. The resulting product is immobilized via the biotin-labeled primer to a streptavidin-coated microplate.
   The detection probe and the hybridization conditions have been optimized for obtaining the highest specificity and sensitivity.

5. The immobilized PCR product is then detected with an antibody against digoxigenin (Anti-DIG-POD) that is conjugated to peroxidase.

6. Finally, the probe is visualized by virtue of peroxidase metabolizing TMB to form a colored reaction product.

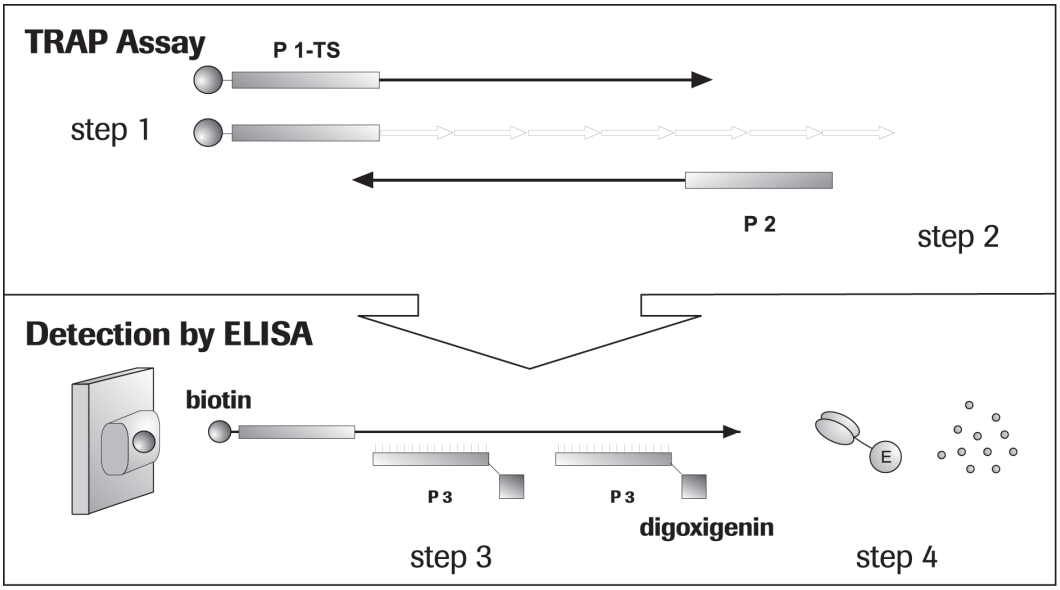

Fig. 3: Principle of the *TeloTAGGG* Telomerase PCR ELISA.

Background information

Telomeres

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 (human repeat sequence: TTAGGG). 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 α is unable to replicate the very 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 appears to be linked to the limited proliferative capacity of normal somatic cells of higher eukaryotes.

Telomerase

It is likely that this activity plays a role in events related to cellular senescence ("mitotic clock"). 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 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 permanent cell lines and tumors. Escaping from the proliferative limitations of cellular senescence, telomerase reactivation might be a prerequisite for the development of malignant tumor cells from somatic cells.

Methods for detecting telomerase activity

The conventional primer-extension based assay for detecting telomerase activity requires large amounts of sample material (cells or tissue), and only allows detection of telomerase with limited sensitivity. Those disadvantages have been overcome by the Telomeric Repeat Amplification Protocol (TRAP), in which the telomerase-reaction product is amplified by PCR. The standard TRAP assay provided full sensitivity only when used with a radioactive label, and visualized by autoradiography after gel electrophoresis, which is both hazardous and time consuming.

5.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Nylon Membranes, positively charged	10 sheets, 20 x 30 cm	11 209 272 001
	20 sheets, 10 x 15 cm	11 209 299 001
	1 roll, 0.3 x 3 m	11 417 240 001
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
RNase, DNase-free	500 µg, 1 ml	11 119 915 001
Streptavidin Conjugates	Streptavidin-AP Conjugate, 1,000 U	11 089 161 001
	Streptavidin-β-Gal Conjugate, 500 U, *Not available in US*	11 112 481 001
	Streptavidin-POD Conjugate, 500 U	11 089 153 001
Blocking Reagent	50 g	11 096 176 001

6.4. Trademarks

All product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

6.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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