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

*Telo**TAGGG*** Telomere Length Assay

Version: 11
Content Version: December 2020

Nonradioactive chemiluminescent assay to determine telomere length

Cat. No. 12 209 136 001 1 kit
50 reactions

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	yellow	*Telo*TAGGG Telomere Length Assay, Hinf I	For digestion of genomic DNA.	1 bottle, 30 µl (40 U/µl)
2	yellow	*Telo*TAGGG Telomere Length Assay, Rsa I	For digestion of genomic DNA.	1 bottle, 30 µl (40 U/µl)
3	violet	*Telo*TAGGG Telomere Length Assay, Digestion buffer, 10x conc.	For digestion of genomic DNA.	1 bottle, 200 µl
4	colorless	*Telo*TAGGG Telomere Length Assay, Water, nuclease-free	For digestion of genomic DNA and gel electrophoresis.	1 bottle, 1.1 ml
5	green	*Telo*TAGGG Telomere Length Assay, Control DNA	Purified genomic DNA from immortal cell lines.	1 bottle, 150 µl
6	green	*Telo*TAGGG Telomere Length Assay, DIG Molecular weight marker	Fragments: 21226, 8576, 7427, 6106, 5148, 4973, 4899, 4268, 3639, 3530, 2799, 2027, 1953, 1904, 1882, 1584, 1515, 1482, 1375, 1164, 992, 947, 831, 718, 710, 564, 492, 359, 125, 81 bp.	1 bottle, 40 µl
7	blue	*Telo*TAGGG Telomere Length Assay, Loading buffer, 5x conc.	For digestion of genomic DNA and gel electrophoresis.	1 bottle, 400 µl
8	colorless	*Telo*TAGGG Telomere Length Assay, DIG Easy Hyb Granules	For hybridization.	2 bottles, 100 ml each
9	white	*Telo*TAGGG Telomere Length Assay, Telomere probe	DIG-labeled, telomere-specific hybridization probe.	1 bottle, 25 µl
10	blue	*Telo*TAGGG Telomere Length Assay, Washing buffer, 10x conc.	For washing steps.	2 bottles, 100 ml each
11	green	*Telo*TAGGG Telomere Length Assay, Maleic acid buffer, 10x conc.	For preparation of Blocking solution.	1 bottle, 100 ml
12	colorless	*Telo*TAGGG Telomere Length Assay, Blocking buffer, 10x conc.	For preparation of Blocking solution.	1 bottle, 100 ml
13	red	*Telo*TAGGG Telomere Length Assay, Anti-DIG-AP	Fab fragments of a polyclonal antibody from sheep, conjugated to alkaline phosphatase (AP).	1 bottle, 60 µl (0.75 U/µl)
14	black	*Telo*TAGGG Telomere Length Assay, Detection buffer, 10x conc.	For chemilunimescence detection.	1 bottle, 75 ml
15	white (with dropper)	*Telo*TAGGG Telomere Length Assay, Substrate solution	▪ Ready-to-use solution. ▪ Contains CDP-*Star*, a highly sensitive chemiluminescence substrate.	1 bottle, 30 ml

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	yellow	Hinf I	Store in aliquots at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	yellow	Rsa I	
3	violet	Digestion buffer, 10x conc.	
4	colorless	Water, nuclease-free	Store in aliquots at +2 to +8°C.
5	green	Control DNA	Store in aliquots at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
6	green	DIG Molecular weight marker	
7	blue	Loading buffer, 5x conc.	Store in aliquots at +2 to +8°C.
8	colorless	DIG Easy Hyb Granules	Store in aliquots at +15 to +25°C.
9	white	Telomere probe	Store in aliquots at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
10	blue	Washing buffer, 10x conc.	Store in aliquots at +15 to +25°C.
11	green	Maleic acid buffer, 10x conc.	
12	colorless	Blocking buffer, 10x conc.	Store in aliquots at +2 to +8°C.
13	red	Anti-DIG-AP	
14	black	Detection buffer, 10x conc.	Store in aliquots at +15 to +25°C.
15	white	Substrate solution	Store in aliquots at +2 to +8°C.

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Microcentrifuge
- Gel electrophoresis equipment
- Blotting equipment
- UV-crosslinker or transilluminator
- Shaking water bath
- Orbital shaker
- Hybridization oven
- Imaging device or X-ray film or Lumi film*
- Imaging system to quantify chemiluminescence signals

For preparation of kit working solutions

- Premixed TAE Buffer, 10x*
- HCl solution
- Denaturation solution
- Neutralization solution
- Premixed SSC Buffer, 20x*
- Autoclaved, double-distilled water
- Sodium dodecyl sulfate (SDS)*

For preparation of genomic DNA

- DNA Isolation Kit for Cells and Tissues*
- High Pure PCR Template Preparation Kit*

For Southern blotting and chemiluminescence detection

- Agarose MP*
- Nylon Membranes, positively charged*
- Hybridization Bags*
- Lumi-Film Chemiluminescent Detection Film*

1.4. Application

The *Telo*TAGGG Telomere Length Assay is designed to be used in the following life science research applications:

- Sensitive detection of telomeric DNA (telomeric sequence: TTAGGG) from cell cultures and other biological samples.
- Determination of the telomere length of these samples.

The kit provides all components required to perform digestion of purified genomic DNA and hybridization and detection of telomeres.

1.5. Preparation Time

Assay Time

Step	Approximate Duration [hours]
Genomic DNA digestion	2
Gel electrophoresis and Southern blotting	10
Hybridization and chemiluminescence detection	6
Total assay time	**18**

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The *Telo*TAGGG Telomere Length Assay can be used with:
- Cell cultures
- Scientific biopsy material
- Other biological research samples.

General Considerations

Precautions

- SDS used in the assay is toxic or corrosive and must be handled with care.
- Perform the entire assay under nuclease-free conditions.
- Use only autoclaved, double-distilled water.
- Prepare appropriate aliquots of the kit solutions and keep them separated from other laboratory reagents.
- Use autoclaved or heat-sterilized labware, such as pipettes, pipette tips, and reactions vials.
- Wear gloves at all times.

Number of tests

Up to 50 reactions can be performed, including 10 control reactions.

Safety Information

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
1	TAE buffer	0.04 M Tris-acetate, 0.001 M EDTA, pH 8.0	Store at +15 to +25°C.
2	HCl solution	▪ 0.25 M HCl ▪ For a 200 cm^2 blot, approximately 250 ml of solution is needed.	
3	Denaturation solution	▪ 0.5 M NaOH, 1.5 M NaCl ▪ For a 200 cm^2 blot, approximately 500 ml of solution is needed.	
4	Neutralization solution	▪ 0.5 M Tris-HCl, 3 M NaCl, pH 7.5 ▪ For a 200 cm^2 blot, approximately 500 ml of solution is needed.	
5	20x SSC	3 M NaCl, 0.3 M sodium citrate, pH 7.0	
6	2x SSC	Dilute 20x SSC (Solution 5) 1:10 with autoclaved, double-distilled water.	
7	DIG Easy Hyb Granules	Reconstitute the granules (Bottle 8) with 64 ml autoclaved, double-distilled water and incubate at +37°C until completely reconstituted.	Prepare solution several hours before use. Store at +15 to +25°C for 3 months.
8	Stringent wash buffer I	2x SSC, 0.1% SDS	Store at +15 to +25°C.
9	Stringent wash buffer II	0.2x SSC, 0.1% SDS	
10	Washing buffer, 1x	▪ Thaw Washing buffer, 10x conc. (Bottle 10) and mix homogeneously. ▪ Dilute an appropriate volume of Washing buffer, 10x conc. 1:10 with autoclaved, double-distilled water.	
11	Blocking solution, 1x	▪ Thaw Blocking buffer, 10x conc. (Bottle 12) and mix homogeneously. ▪ Dilute an appropriate volume of Blocking buffer, 10x conc. 1:10 with Maleic acid buffer, 1x (Solution 12).	Prepare immediately before use. ⚠ ***Do not store.***
12	Maleic acid buffer, 1x	▪ Thaw Maleic acid buffer, 10x conc. (Bottle 11) and mix homogeneously. ▪ Dilute an appropriate volume of Maleic acid buffer, 10x conc. 1:10 with autoclaved, double-distilled water.	Store at +15 to +25°C.
13	Anti-DIG-AP, working solution	▪ To reduce background by aggregated antibody, spin bottle for 5 minutes at 13,000 rpm before use. ▪ Dilute an appropriate volume of Anti-DIG-AP (Bottle 13) with Blocking solution, 1x (Solution 11) to a final concentration of 75 mU/ml (1:10,000).	Prepare immediately before use. ⚠ ***Do not store.***
14	Detection buffer, 1x	▪ Thaw Detection buffer, 10x conc. (Bottle 14) and mix homogeneously. ▪ Dilute an appropriate volume of Detection buffer, 10x conc. 1:10 with autoclaved, double-distilled water.	Store at +15 to +25°C.

2.2. Protocols

Detection of telomeric DNA using genomic DNA of *in vitro* cell cultures and scientific biopsy material

⚠ ***To avoid carryover contamination, transfer the required volumes of each solution for one experiment into a fresh tube instead of directly pipetting from stock solutions.***

Preparation of genomic DNA

1. Isolate genomic DNA using standard protocols or kits*.

2. To ensure maximum efficiency in the digestion of genomic DNA, spin the genomic DNA solution in a centrifuge for 5 minutes at maximum speed.

3. Transfer the clarified supernatant containing the DNA to a fresh tube and use it in the digestion reaction.

Genomic DNA digestion

⚠ ***Keep all solutions and perform pipetting on ice.***

1. Prepare a Hinf I/Rsa I enzyme mixture by mixing equal volumes of Hinf I (Bottle 1) and Rsa I (Bottle 2).
   – This results in an enzyme concentration of 20 U/µl for each enzyme.

2. 1 µl of the enzyme mixture is needed for each sample to be tested and for the Control DNA (Bottle 5).
   i Prepare enough enzyme mixture to digest all the samples to be analyzed in this experiment. For example, if you are analyzing 8 samples and 2 positive controls, mix 6 µl Hinf I (Bottle 1) and 6 µl Rsa I (Bottle 2).

3. In a reaction vial, dilute 15 µl (= 1.5 µg) Control DNA (Bottle 5) with 2 µl nuclease-free Water (Bottle 4) to produce a final volume of 17 µl.

4. For each sample, dilute 1 to 2 µg of purified genomic DNA with nuclease-free Water (Bottle 4) to a final volume of 17 µl in a reaction vial.

5. To each genomic DNA sample, add 2 µl of Digestion buffer, 10x conc. (Bottle 3), and 1 µl of Hinf I/Rsa I enzyme mixture and mix.

6. Incubate the reaction mixture for 2 hours at +37°C.

7. To stop the reaction, add 5 µl of gel electrophoresis Loading buffer, 5x conc. (Bottle 7) and quickly spin the reaction vials.

Southern blotting

Gel electrophoresis

i Separation of digested DNA is done by agarose gel electrophoresis following standard protocols.

1. Prepare a 0.8% agarose gel, approximately 15 cm in length in 1x TAE buffer (Solution 1) using highly pure, nucleic-acid grade agarose such as Agarose MP.

2. In a reaction vial, mix 4 µl DIG Molecular weight marker (Bottle 6), 12 µl nuclease-free Water (Bottle 4), and 4 µl 5x Loading buffer (Bottle 7); quickly spin the vial.

3. Load 1 to 2 µg of each digested DNA sample onto a lane of the gel.
   i To obtain valid quantitative analysis of telomere length, each sample should contain the same amount of DNA.

4. To ensure accurate length measurement, load one aliquot (10 µl) of diluted DIG Molecular weight marker to each side of the gel (flanking the sample lanes).

5. Run gel at 5 V/cm in 1x TAE buffer (Solution 1) until the Bromophenol blue tracking dye is separated, approximately 10 cm from the starting wells (total run time 2 to 4 hours).

Blotting

Southern transfer of the digested DNA can be done by either capillary transfer or vacuum transfer using 20x SSC transfer buffer or alkaline transfer buffers. However, maximum transfer efficiency and sensitivity are obtained with positively charged nylon membranes using capillary transfer and 20x SSC transfer buffers.

Perform all incubation steps with gentle agitation.

1. Submerge the gel in HCl solution (Solution 2) and agitate for 5 to 10 minutes at +15 to +25°C until the Bromophenol blue stain changes color to yellow.

2. Rinse the gel 2 times with water.

3. Submerge the gel in the Denaturation solution (Solution 3) for 2 × 15 minutes at +15 to +25°C.

4. Rinse the gel 2 times with water.

5. Submerge the gel in the Neutralization solution (Solution 4) for 2 × 15 minutes at +15 to +25°C.
   Wear unpowdered rubber gloves and handle the membrane with forceps only at the edges.

6. Prepare all of the equipment necessary for performing a Southern blot.

7. Blot the digested DNA from the gel to the nylon membrane by capillary transfer at +15 to +25 °C using 20x SSC (Solution 5) as a transfer buffer.
   Transfer for 6 hours gives sufficient sensitivity in most applications. However, for maximum sensitivity and reproducibility of the results, perform overnight blotting.

8. After Southern transfer, fix the transferred DNA on the wet blotting membrane by UV-crosslinking (120 mJ) or by baking the membrane at +120°C for 20 minutes.

9. Wash the blotting membrane 2 times with 2x SSC (Solution 6).

10. If not used immediately for the hybridization and chemiluminescence detection step, air dry the blotting membrane and store at +2 to +8°C.

Hybridization and chemiluminescence detection

The volumes recommended in the following steps are based on a membrane size of 200 cm². Adjust the volumes accordingly if other membrane sizes are used. Maintain the hybridization and stringent wash temperatures exactly as shown.

⚠ ***Use clean labware during the detection procedure.***

1. Prewarm approximately 25 ml of DIG Easy Hyb Granules (Solution 7) to +42°C.

2. For prehybridization, submerge the blot in 18 ml of prewarmed DIG Easy Hyb Granules (Solution 7) and incubate for 30 to 60 minutes at +42°C with gentle agitation.

3. Prepare the hybridization solution by adding 1 µl Telomere probe (Bottle 9) per 5 ml fresh prewarmed DIG Easy Hyb Granules (Solution 7) and mix.
   – At least 6.5 ml of hybridization solution will be needed per 200 cm² blotting membrane.

4. Discard prehybridization solution completely and immediately add hybridization solution to the membrane.

5. Incubate for 3 hours at +42°C with gentle agitation and discard the hybridization solution.

6. Wash the membrane twice with Stringent wash buffer I (Solution 8) for 5 minutes at +15 to +25°C, with gentle agitation.

7. Wash the membrane twice with prewarmed Stringent wash buffer II (Solution 9) in a heated water bath for 15 to 20 minutes at +50°C with gentle agitation.

8. Rinse membrane in at least 100 ml 1x Washing buffer (Solution 10) for 1 to 5 minutes at +15 to +25°C with gentle agitation.

9. Incubate the membrane in 100 ml freshly prepared 1x Blocking solution (Solution 11) for 30 minutes at +15 to +25°C with gentle agitation.

10. Incubate the membrane in 50 to 100 ml Anti-DIG-AP, working solution (Solution 13) for 30 minutes at +15 to +25°C with gentle agitation.
    To prepare Anti-DIG-AP, working solution (Solution 13), see section, **Working Solution**.

11. Wash the membrane twice with 100 ml 1x Washing buffer (Solution 10) for 15 minutes at +15 to +25°C with gentle agitation.

12. Incubate the membrane in 100 ml of 1x Detection buffer (Solution 14) for 2 to 5 minutes at +15 to +25°C with gentle agitation.

13. Discard Detection buffer (Solution 14) and remove excess liquid from the membrane by placing the membrane, DNA side up, on a sheet of absorbent paper.
    Do not let the membrane dry.

14. Immediately place the wet membrane, DNA side facing up, on an opened Hybridization Bag and very quickly apply approximately 40 drops (approximately 3 ml) Substrate solution (Bottle 15) to the membrane.

15. Immediately cover the membrane with the second sheet of the Hybridization Bag, being careful to spread the substrate solution homogeneously over the membrane without trapping air bubbles.

16. Incubate the membrane for 5 minutes at +15 to +25°C.

17. Squeeze out excess Substrate solution and seal the edges of the Hybridization Bag.

18. Expose to an imaging device or to X-ray film or Lumi-Film for 20 minutes at +15 to +25°C.

Luminescence continues for at least 24 hours and signal intensity will increase during the first hours.

2.3. Parameters

Detection range

Detects telomeres from a variety of organisms. Average telomeric length 100 bp to 20 kbp.

Sensitivity

Detects telomeric DNA from 1 µg genomic DNA.

3. Results

Data analysis

Telomere length of human cell samples may range over one order of magnitude. Even within a population of cell lines and on a single cell level, considerable heterogeneity of telomere length was shown. Therefore, analyzing a population of cells provides the average telomere length of the telomeres in the sample, indicated by a smear at a specific length compared to the molecular weight standard. As described in section, **Principle**, the TRFs comprise not only the variable terminal array but also the subtelomeric region, which has been estimated to be about 3 to 4 kbp in length. After exposure of the blot to an X-ray film, an estimate of the mean TRF length can be obtained by visually comparing the mean size of the smear to the molecular weight marker. However, for quantitative measurements of mean TRF length, the chemiluminescence signals should be scanned by an imaging system.

Calculation method

Mean TRF length has been defined according to the following formula, where OD_i is the chemiluminescent signal and L_i is the length of the TRF at position i (Fig 1). The calculation takes into account the higher signal intensity from larger TRFs due to multiple hybridizations of the telomere-specific hybridization probe.

$$\overline{TRF} = \frac{\Sigma (OD_i)}{\Sigma (OD_i / L_i)} \qquad (1)$$

Fig. 1: Calculation of the mean TRF length.

Calculation

The mean TRF length can be calculated by scanning the exposed X-ray film with a densitometer. To obtain reliable results, the signal strength must be within the linear range of the X-ray film.

1. Scan the exposed X-ray film with a densitometer.

2. Overlay each sample lane of the scanned image with a grid (Fig. 2).
   – The height of the individual squares of the grid determines the resolution of the TRF length calculation. Typically, >30 squares per lane are recommended.

3. For background subtraction, select several grid squares in each lane where no telomere-specific signal is found and which are representative of the background in that lane.
   – Signals from these squares should be averaged and subtracted from each square that contains DNA.

4. For each square that contains DNA, determine the signal (OD_i) and the corresponding length L_i where OD_i is the total signal intensity within that square and L_i is the molecular weight at the midpoint of that square.

5. Calculate the mean TRF length using the formula.

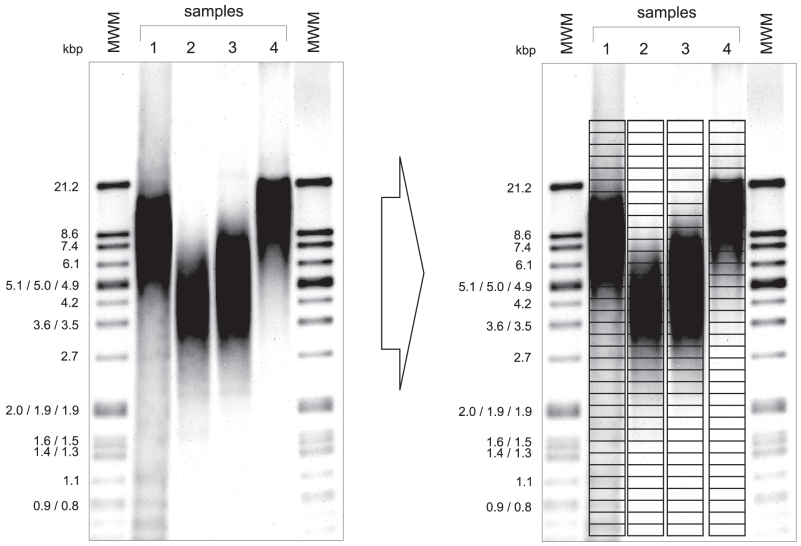

Fig. 2: Chemiluminescent detection of TRFs. Immortalized cell lines (samples 1 to 4) were analyzed for telomere length using the *Telo*TAGGG Telomere Length Assay. The data was analyzed as described, resulting in the following mean TRF length of the various samples:
Sample 1: 7.4 kb
Sample 2: 3.9 kb
Sample 3: 4.6 kb
Sample 4: 10.2 kb

Typical results

The following figure shows typical results. It demonstrates the specificity and sensitivity of the assay for detecting telomeric DNA in different immortalized cell lines (Fig. 3). The mean TRF length can be calculated according to the formula as described in section, **Data analysis**.

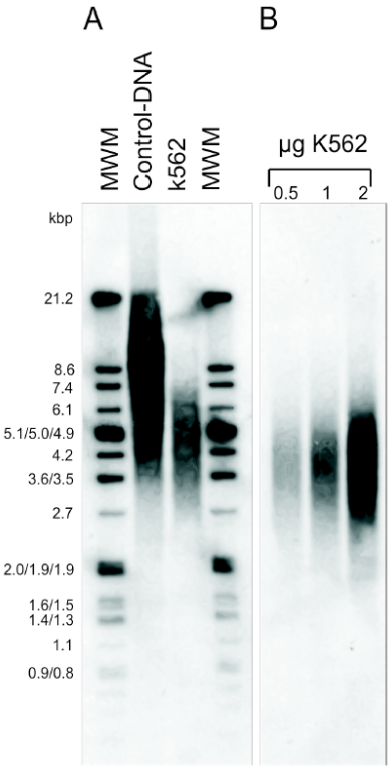

Fig. 3: Specificity (A) and Sensitivity (B) of the *Telo*TAGGG Telomere Length Assay. Immortalized cell lines were analyzed for telomere length using the *Telo*TAGGG Telomere Length Assay.

4. Troubleshooting

Observation	Possible cause	Recommendation
Membrane background too high.	Membrane is not uniformly covered by the detection reagents.	Use sufficient amount of the detection reagents.
	Concentration of the telomere probe in the hybridization solution is too high.	Reduce concentration of the telomere probe.
	Aggregates in the tube.	Quickly spin the Anti-DIG-AP before use.
	Contamination of working solutions.	Check diluted working solutions for bacterial contamination.
Weak or no signals.	If DIG Molecular weight marker is hardly or not at all visible, inefficient transfer of the restricted DNA to the membrane occurred.	Increase the DNA amount loaded onto the gel, up to 7.5 µg/lane.
		Repeat the entire experiment with the Control DNA supplied with the kit.
Multiple bands above the telomere signal.	Inadequate purity of genomic DNA.	Prolong digestion of DNA.
		Prepare fresh reagents and repeat experiment.
		Prolong digestion of the genomic DNA.
	If multiple bands also occur in Control DNA lane, reagents may be contaminated.	Prepare fresh reagents and repeat experiment.
Multiple bands below the telomere signal.	The temperature is too low.	Carefully check the temperature for the stringent wash step II.
	Undesired hybrids were not disrupted.	During detection, increase time allowed for each stringent wash.
DIG marker shows more bands than expected.	Marker was incubated at +65°C for 10 minutes.	Do not heat DIG Molecular weight marker prior to loading on the agarose gel.

5. Additional Information on this Product

5.1. Test Principle

An overview of the test principle is shown below and in Figure 4.

1. Isolation of genomic DNA.

2. Purified genomic DNA is digested by an optimized mixture of frequently cutting restriction enzymes. The sequence specificity of these enzymes ensures that telomeric DNA and sub-telomeric DNA is not cut, while non-telomeric-DNA is digested to low molecular weight fragments.

3. Separation of DNA fragments.

4. Southern transfer of DNA fragments.
   – Following DNA digestion, the DNA fragments are separated by gel electrophoresis and transferred to a nylon membrane by Southern blotting.

5. Blotted DNA fragments are hybridized with a telomere-specific, digoxigenin (DIG)-labeled hybridization probe.

6. Incubation with Anti-DIG-AP.

7. Chemiluminescence detection.
   – Alkaline phosphatase on the antibody metabolizes CDP-*Star*, a highly sensitive chemiluminescent substrate, producing a visible signal that indicates the location of the immobilized telomere probe, or Telomere Restriction Fragment (TRF) on the blot.

8. TRF analysis.
   – The average TRF length can be determined by comparing the location of the TRF on the blot relative to a molecular weight standard.

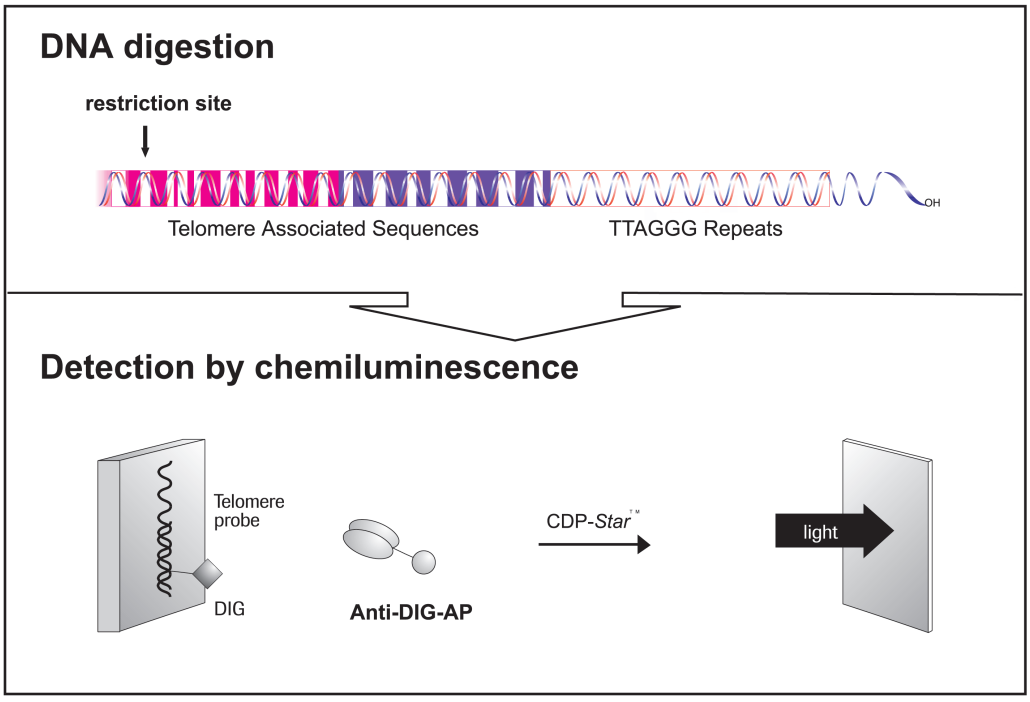

Fig. 4: Test principle

Background information

Telomeres

Telomeres, the specialized DNA/protein structures located at the end of eukaryotic chromosomes, consist of small, tandemly repeated DNA sequences. Numerous telomere sequences have been identified, which show very little sequence variation, even between phylogenetically divergent organisms such as *Tetrahymena* (sequence: TTGGGG) and human (sequence: TTAGGG). Despite the homology in its sequences, telomeric DNA shows a significant variation with respect to its length, for example, the length of the simple repeat region can range from <50 bp in *Euplotes* to >100 kbp in mice. The assay should only be used to detect expected telomeric lengths of 100 bp to 20 kbp because this is the range of the DIG Molecular weight marker included in this product.

Telomere hypothesis

As DNA polymerase α is unable to replicate the very ends of linear DNA, it was proposed that chromosomal ends progressively shorten with each replication cycle (called the "end-replication problem"). 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"). Since germline cells, stem cells, and tumor cells exhibit a prolonged or even infinite life span, it was proposed that these cells must possess a particular mechanism for maintaining telomere length.

Telomerase

Maintaining stable telomere length is associated with activation of telomerase. This enzyme is a ribonucleoprotein that compensates for the loss of telomeric DNA by adding repeat sequences to the chromosome ends using its intrinsic RNA component as a template for DNA synthesis. The genes encoding its RNA subunit and the catalytic protein subunit have been cloned from a variety of species, including humans. 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. Additional yeast and mouse strains that lack any of these telomerase components, do not have detectable telomerase activity and undergo telomere shortening and loss of viability, indicating that telomerase expression is required to maintain telomere length *in vivo*.

Telomere function

Telomeres play an essential role in the stable maintenance of the eukaryotic chromosome within a cell by specifically binding to structural proteins. These proteins cap the ends of linear chromosomes, preventing nucleolytic degradation, end-to-end fusion, irregular recombination, and other events that are normally lethal to a cell. Additionally, telomeres are involved in nuclear architecture and interact with other proteins to repress the expression of adjacent genes. Analysis of telomere length in human peripheral blood mononuclear cells reveals that telomere length decreases with increased age in the donor, reflecting the replicative history of those cells. In several disorders, such as Down's syndrome, ataxia telangiectasia, and during HIV infection, accelerated telomere loss has been described, suggesting that the reduction in telomere length may be related to the immune dysfunction in these disorders. This kit is intended to increase scientific knowledge about these relationships.

Methods for detecting telomeres and measuring telomere length

Various methods have been described to detect telomeres and to measure telomere length. The standard method to assess telomere length utilizes Southern analysis of terminal restriction fragments (TRF) obtained by digestion of genomic DNA using frequently cutting restriction enzymes. The TRFs obtained contain DNA with uniform telomeric (TTAGGG) repeats as well as degenerate repeats other than at the distal end of the chromosome (subtelomeric region). After digestion, the DNA fragments are separated by gel electrophoresis and blotted. TRFs are visualized directly or indirectly by hybridization with labeled oligonucleotides complementary to the telomeric repeat sequence. Finally, the size distribution of the TRFs can be compared to a DNA length standard.

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.
Consumables		
Hybridization Bags	50 bags, 25 cm x 23 cm	11 666 649 001
Reagents, kits		
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
Buffers in a Box, Premixed SSC Buffer, 20x	4 l	11 666 681 001
Buffers in a Box, Premixed TAE Buffer, 10x	4 l	11 666 690 001
Lumi-Film Chemiluminescent Detection Film	100 films, 8 x 10 inches, 20.3 x 25.4 cm	11 666 657 001
	100 films, 7.1 x 9.4 inches, 18 x 24 cm, *Not available in US*	11 666 916 001
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
Agarose MP	100 g	11 388 983 001
	500 g	11 388 991 001
High Pure PCR Template Preparation Kit	1 kit, up to 100 purifications	11 796 828 001
DNA Isolation Kit for Cells and Tissues	1 kit, 10 isolations	11 814 770 001

6.4. Trademarks

DIG EASY HYB is a trademark of Roche.
All other 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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