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Telomere PNA Kit/FITC for Flow Cytometry

A convenient flow-FISH kit to measure telomere length with reproducible and reliable results

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Measuring telomere length with flow-FISH for rapid examination of a large number of cells

Telomere length depends on the age of the cell and the number of times the cell has divided, being considered a marker of cellular replicative history and remaining replication capability. Therefore, the assessment of telomere length is of great significance as a research tool in cancer studies, telomeropathies and other telomere biology disorders.

The relevance of telomeres and telomerase

Telomeres in all vertebrates are composed of a tandem sequence of six nucleotides (TTAGGG) repeated up to several thousand times. Telomere length and telomerase activity are important factors in the pathobiology of several human diseases. During the process of DNA synthesis and cell division, telomeres shorten as a result of the incomplete replication of linear chromosomes. This progressive telomere shortening is one of the molecular mechanisms underlying aging, chromosome senescence and loss of cell viability.

Telomerase is a ribonucleoprotein enzyme that functions as a reverse transcriptase positively regulating telomere length by elongating the ends. It has been proven that activation of telomerase is necessary for extension of the life-span of human cells by avoiding telomere shortening (1). In somatic cells, the activity of telomerase is usually diminished after birth so that the telomere length is gradually shortened with cell divisions triggering cellular senescence. In embryonic stem cells, telomerase is activated and maintains telomere length and cellular immortality. However, the level of telomerase activity is low or absent in the majority of stem cells regardless of their proliferative capacity. Almost 90% of human primary tumors express telomerase, while the cells of most normal tissues lack this enzyme activity. Therefore, telomerase activity and the preservation of telomere length are important factors in the study of cancerous processes.

Agilent's Telomere PNA Kit/FITC for Flow Cytometry is recommended for use on nucleated hematopoietic cells from all vertebrates. When using this kit, the cell line 1301 is recommended as control cells. The 1301 cell line is very easy to distinguish from most other cell types because of its characteristic features: tetraploidy and long telomeres. Other cell types that are easily distinguished from the sample cells can also be used.

Table 1. Comparison between telomere length methods: linear regression and Bland-Altman analysis (2).

		Linear regression			Bland-Altman analysis	
		Equation	R2	p value	Bias ±SD	Limits of agreement: lower and upper
Flow-FISH x TRF	Healthy subjects	y=0.85+0.86x	0.60	<0.0001	0.17±1.03	−1.88/2.24
	Patients	y=2.1+0.67x	0.51	<0.0001	0.0±1.21	−2.41/2.41
qPCR x TRF	Healthy subjects	y=5.2+3.1x	0.35	<0.0001	0.78±1.34	−1.90/3.47
	Patients	y=4.8+2.8x	0.20	0.001	1.15±1.49	−1.84/4.14
Flow-FISH x qPCR	Healthy subjects	y=5.6+2.7x	0.33	<0.0001	−0.6±1.27	−3.16/1.94
	Patients	y=5.6+1.2x	0.10	0.08	−1.15±1.65	−4.45/2.15

Measuring telomere length using flow-FISH technology

Flow-FISH is a method that combines fluorescence in situ hybridization (FISH) with flow cytometry, using labeled peptide nucleic acid (PNA) probes that hybridize to telomere repeats in cells in suspension. Flow-FISH technology applied to measure telomere length permits the rapid examination of a large number of cells with the possibility of using the gating strategy that excludes damaged cells. The flow-FISH technology also serves as a powerful tool for examining telomere length in different cell and tissue types. Flow-FISH technology can be further modified to simultaneously assess and detect cell phenotypes and intracellular cytokines with the assessment of telomere length. The advantages of flow-FISH technology permit large-scale screening of samples for changes in telomere length in several research laboratories with reproducible and reliable results.

A study (2) was conducted to compare methods for the measurement of telomeres. In the study, the gold standard of terminal restriction fragment (TRF) analysis by Southern blot was compared to flow-FISH and qPCR to measure telomere length of human peripheral blood leukocytes. Parameters such as intra- and inter-assay variability, sensitivity and specificity of flow-FISH and qPCR were evaluated. The study demonstrated that flow-FISH displayed a better correlation with the gold standard TRF analysis by Southern blot in comparison to qPCR (Table 1). Apart from being more accurate, precise, and reproducible, flow-FISH also showed better sensitivity and specificity especially in patients with shorter telomeres.

Agilent's Telomere PNA Kit/FITC for Flow Cytometry

Telomere PNA Kit/FITC for Flow Cytometry is based on the flow-FISH principle and is intended for the detection of telomeres in nucleated hematopoietic cells using a fluorescein-conjugated peptide nucleic acid (PNA) probe. The kit may be used for the detection of telomeres from all vertebrate nucleated hematopoietic cells. Results are evaluated by flow cytometry using a light source with excitation at 488 nm. The probe does not recognize subtelomeric sequences, and in contrast to traditional telomere restriction fragment (TRF) measurements, the kit therefore allows an estimation of the telomere length without inclusion of subtelomeres.

The kit has been designed so that post-hybridization treatments are kept to a minimum and formamide washes are avoided. The kit uses PNA probes, which are superior to DNA probes in terms of sensitivity and specificity (3). The method is optimal for estimation of telomere length, as the fluorescence intensity of the cells is directly correlated to the length of the telomere (4, 5).

Procedure and principle

Day 1

Pre-treatment
- Wash sample cells & control cells in PBS
- Enumerate cells in Bürker chamber
- Mix sample cells & control cells
- Centrifuge at 500g for 5 minutes
- Remove supernatant by suction/aspiration

Denaturation & hybridization
- Hybridize controls and samples with hybridization solution (Vial 1) & PNA probe/FITC hybridization solution (Vial 2)
- Incubate on a heating block at 82 °C for 10 min
- Vortex and incubate overnight in the dark at room temperature

Day 2

Washing
- Wash with diluted wash solution (Vial 3) and mix
- Incubate on a heating block at 40 °C for 10 min
- Mix and centrifuge at 500g for 5 minutes and decant the supernatant

DNA staining
- Add DNA staining solution and mix
- Incubate in the dark at 2-8 °C for 2-3 hours

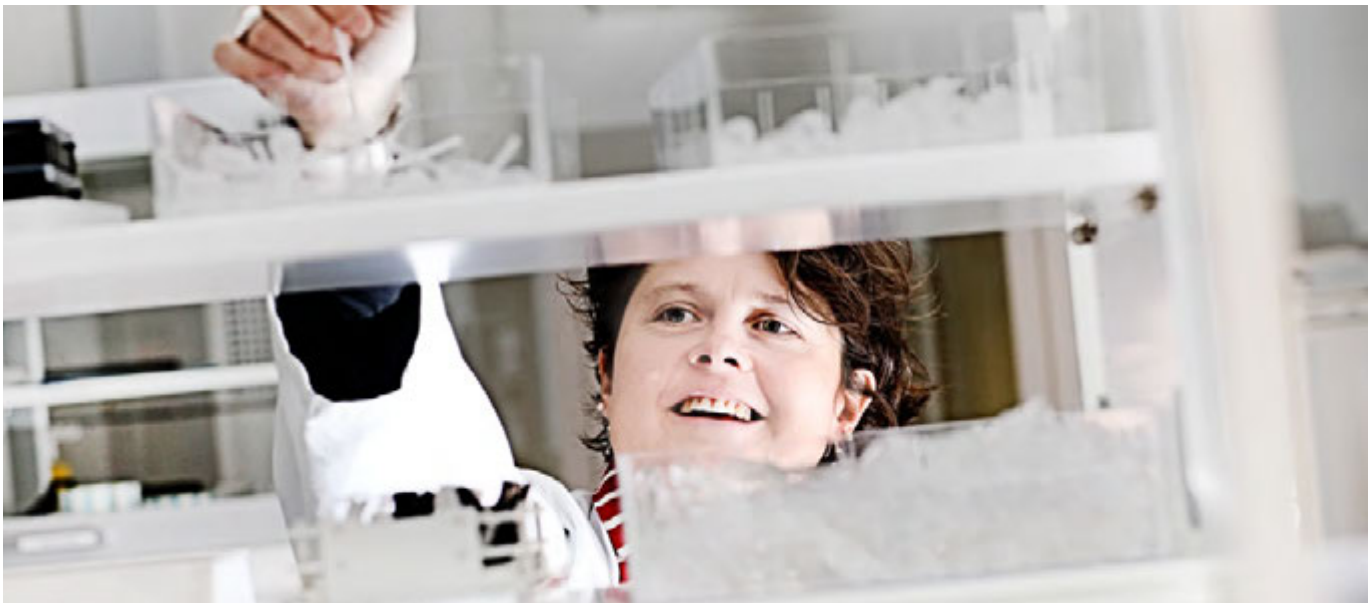
Analysis
- Analyze on flow cytometer and interpret results using RTL formula

The data from the flow cytometric analysis can be used for determination of a relative telomere length (RTL). RTL is calculated using the formula which provides a value representing the telomere length of sample cells compared to telomere length of control cells. DNA index of sample and control cells should be determined using techniques preferred by the user.

Calculation of relative telomere length

Relative telomere length (RTL) =

$$\frac{\text{(Mean FL1 sample cells with probe – Mean FL1 sample cells without probe) x DNA index of control cells x 100}}{\text{(Mean FL1 control cells with probe – Mean FL1 control cells without probe) x DNA index of sample cells}}$$



Human mononuclear cells isolated from blood by gradient centrifugations

The cells were mixed 1:1 with control cells (1301 cell line). As shown in the FL3-height versus FL1-height dot plots (Figures A and B), it is possible to identify cells in the $G_{0/1}$-phase and set gates around these populations (5). Statistical data on these cells are then used for calculation of RTL of the sample cells compared to the control cells. This RTL-value indicates the average telomere fluorescence per chromosome/genome in the sample cells.

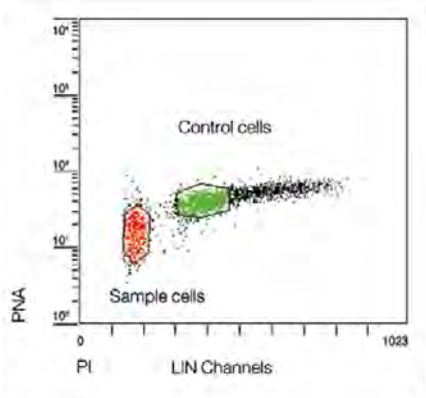


Figure A. Dot plot of FL1-height versus FL3-height of cells hybridized with Hybridization Solution from Telomere PNA Kit/FITC without the telomere PNA probe. Gates are set around cells in the $G_{0/1}$-phase for both sample cells (human mononuclear cells isolated from blood) and control cells (1301 cell line).

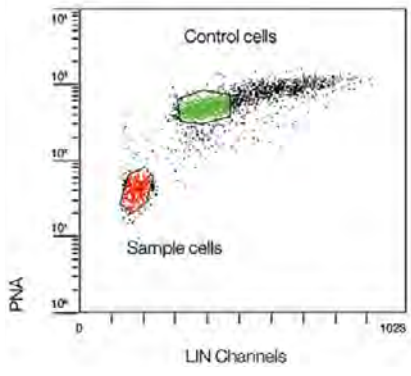


Figure B. Dot plot of FL1-height versus FL3-height of cells hybridized with the telomere PNA probe/FITC in Hybridization Solution from Telomere PNA Kit/FITC. Gates are set around cells in the $G_{0/1}$-phase for both sample cells (human mononuclear cells isolated from blood) and control cells (1301 cell line).

Advantages of Agilent's Telomere PNA Kit/FITC for Flow Cytometry

- Allows an estimation of the telomere length without inclusion of subtelomeres, in contrast to traditional telomere restriction fragment (TRF) measurements;
- Has been optimized, so post-hybridization treatments are kept to a minimum and formamide washes are reduced;
- Uses a PNA probe, which is superior to DNA probes in terms of sensitivity and specificity;
- Is suitable for large-scale screening of samples in several laboratories with reproducible and reliable results;
- Provides a more accurate, precise and reproducible technique when compared with TRF and qPCR to measure telomere length.

Kit contents

Vial 1	Hybridization Solution Without probe, containing 70% formamide 12 mL, ready-to-use
Vial 2	Telomere PNA Probe/FITC FITC-conjugated PNA probe in hybridization solution, containing 70% formamide 12 mL, ready-to-use
Vial 3	Wash Solution 10 x concentrated buffer for post-hybridization washing of samples 20 mL, dilute in pure water
Vial 4	DNA-Staining Solution 10 x buffer with propidium iodide and RNase A for staining of DNA before flow cytometric analysis 4 mL, dilute in pure water

References

1. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu C-P, Morin GB, et al. Extension of life-span by introduction of telomerase into normal human cells. Science 1998;279:349-52.
2. Gutierrez-Rodrigues F, Santana-Lemos BA, Scheucher PS, Alves-Paiva RM, Calado RT. Direct comparison of flow-FISH and qPCR as diagnostic tests for telomere length measurement in humans. PLoS ONE 2014;9(11).
3. Lansdorp PM, Erwoerd NP, van de Rijke FM, Dragowska V, Little M-T, Dirks RW, et al. Heterogeneity in telomere length of human chromosomes. Hum Mol Genet 1996;5:685-91.
4. Hultdin M. Grönlund E, Norrback K-F, Eriksson-Lindström E, Just T, Roos G. Telomere analysis by fluorescence in situ hybridization and flow cytometry. Nucleic Acids Res 1998;26:3651-6.
5. Rufer N, Dragowska W, Thornbury G, Roosnek E, Landsdorp PM. Telomere length dynamics in human lymphocyte subpopulations measured by flow cytometry. Nat Biotechnol 1998;16:743-7.

Ordering information

Product	Size	Code
Telomere PNA Kit/FITC for Flow Cytometry	20 tests	K532711-8

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Learn more:
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