

Telomerase Biomarker Reference Standards for the Detection of Cancer

The enzyme telomerase is expressed in 85% to 90% of all human cancers, but not in normal, non-stem cell somatic tissues at detectable levels. This telomerase expression in a wide variety of cancers makes it a very attractive tumor marker. Clinical assays for telomerase, particularly in easily obtained body fluids, would have great utility as non-invasive and cost-effective methods for the early detection of cancer. Yet, despite the demonstrated utility of telomerase activity assays, there is not yet a universally accepted diagnostic test. One important reason is the lack of a suitable, generally accepted reference material. NIST has developed SRM 2394 Heteroplasmic Mitochondrial DNA Mutation Detection Standard, to meet this need.

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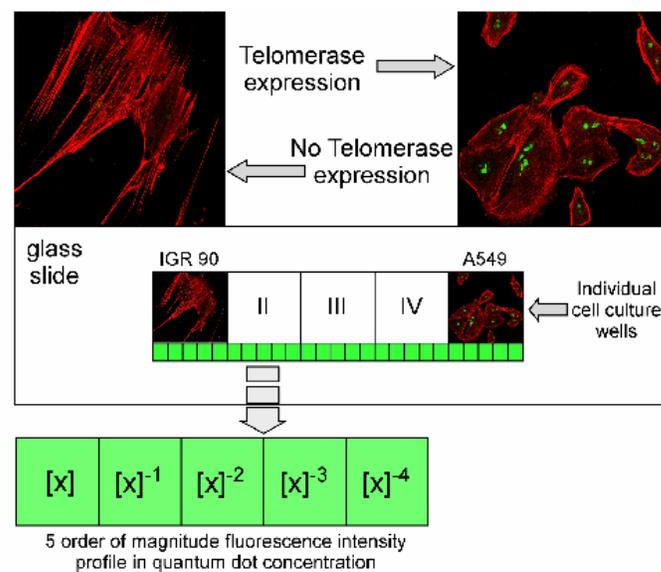
NIST is developing standards for telomerase assays that can be used in a wide range of diagnostic applications. To accomplish this, we are using assay methods recently developed at NIST together with widely used assay methods for the high-throughput analysis of blood samples in cancer patients. One of these methods is the telomerase repeat amplification protocol polymerase chain reaction (TRAP/PCR) assay using capillary electrophoresis and real-time TRAP/PCR as well as reverse transcript (RT)-PCR analysis of telomerase catalytic subunit (hTERT) mRNA. We plan to continue the development of a telomerase SRM with additional methods of characterization, including antibody capture for immuno-histochemistry (IHC) applications and peptide analysis via surface-enhanced laser desorption/ionization (SELDI) mass spectrometry.

In collaboration researchers from NIST's Polymers Division, we are developing a direct bio-imaging method using a standard protein chip and antibody staining to detect telomerase. We have also used 3-D confocal microscopy and fluorescent-labeled telomerase antibodies to directly quantify telomerase in human lung tumor cells and human fibroblast cells (control). The standard IHC protein chip serves as a reference to quantify telomerase concentration at a molecular level and complements the biochemical measurements obtained via TRAP. This combination of measurement of telomerase by tissue staining and TRAP provides a more complete methodology for the quantification of telomerase levels in tissues at various stages of cancer progression.

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Advanced telomerase quantification scheme



Publications:

Jakupciak, J. P., Wang, W., Barker, P. E., Srivastava, S., and Atha, D. H., "Analytical Validation of Telomerase Activity for Cancer Early Detection: TRAP/PCR-CE and hTERT mRNA Quantification Assay for High-Throughput Screening of Tumor Cells." *J. Mol. Diagnostics* (2004) 6, 157-165.

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