

Certificate of Analysis

Product: Affinity Purified Anti-Telomerase catalytic subunit (hTERT) [Rabbit]

Code: 600-401-252

Lot # 19804

Size: 100 µg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 0.44 mg/ml (by UV absorbance at 280 nm)

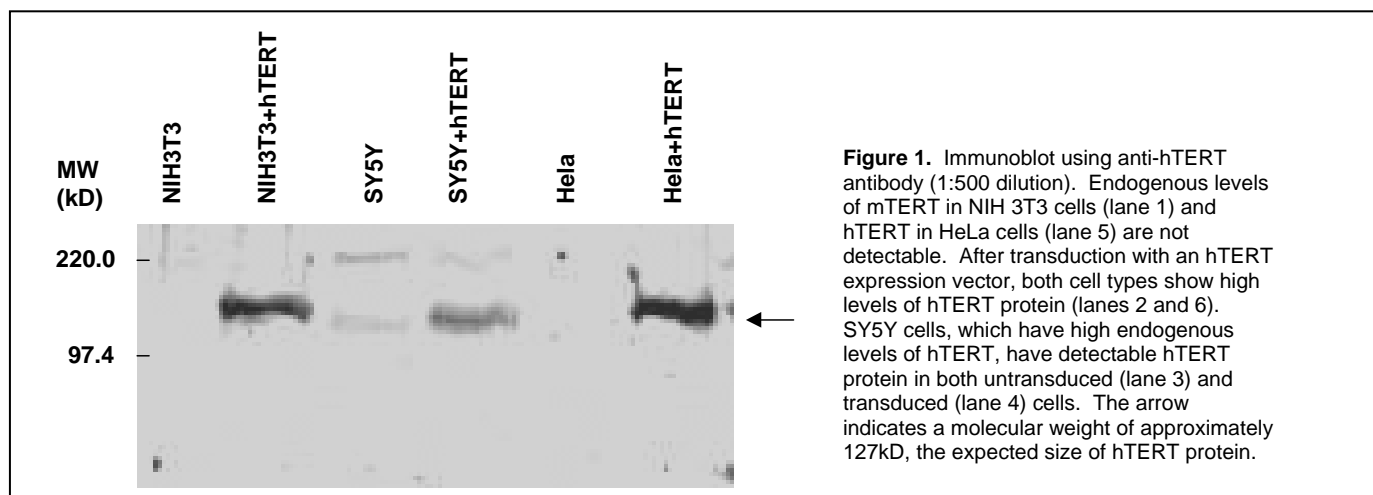
Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C. For extended storage, aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Dilute only prior to immediate use. Expiration date is one (1) year from date of opening.

Background Information: Telomerase is a reverse transcriptase that adds telomeric repeats (TTAGGG)_n to chromosomal ends, compensating for the telomere shortening that occurs with DNA replication. In normal human somatic cells, telomerase is repressed and telomeres progressively shorten, leading to limited lifespan and senescence. Reactivation of telomerase activity is associated with human cancer and cell immortalization. Approximately 85% of human cancers, including breast, prostate, stomach, bladder, colon, and liver cancer, have telomerase activity, whereas most normal somatic cells do not. The specificity of telomerase to human cancer has led to investigations of telomerase activity and expression as a tumor marker. For example, the presence of telomerase activity in human urine has been identified as a marker for human bladder carcinoma. Human telomerase consists of three major subunits: a catalytic protein subunit called hTERT (for human Telomerase Reverse Transcriptase), a template RNA called hTR, and telomerase-associated protein (TEP-1). TERT and hTR are minimally required to reconstitute telomerase activity *in vitro*. In human cells, hTR is constitutively expressed. TERT transcription is a primary mechanism for regulation of telomerase activity.



Application Note(s): This affinity purified antibody has been tested for use in immunoblotting (Figure 1), immunoprecipitation (Figure 2), and immunofluorescence microscopy (Figure 3). In these assays, the antibody detects ectopically-expressed hTERT and high levels of endogenous hTERT. Although it has been reported that this antibody reacts with mouse TERT (mTERT) (see Drissi, *et al.* 2001), the binding to mTERT is considerably weaker and less specific than the binding to hTERT (not shown). A SY5Y cell nuclear extract can be used as a positive control.

Recommended Dilutions: In immunoblot assays, whole cell or nuclear extracts were loaded at a concentration of 100µg protein per well. A working dilution of 1:500 anti-TERT antibody was used followed by a 1:3,000 dilution of horseradish peroxidase- conjugated goat anti-rabbit IgG as the secondary antibody. For immunofluorescence microscopy staining, a working dilution of 1:500 was used followed by a 1:200 dilution of rhodamine-conjugated donkey anti-rabbit IgG as a secondary antibody. Immunoprecipitation was performed using 20 µl of protein A beads and 2 µl of the anti-TERT serum per 1mg protein from cell lysate. A working dilution of 1:500 is also suggested for immunohistochemistry.

Fixation Method: To detect TERT, fix cells in 2% paraformaldehyde (in PBS) for 10'. Wash the slides twice in PBS for 5' each. Permeabilize the cells in 0.5% NP-40 for 10'. Wash as before in PBS. Block the cells using PBG buffer (0.2% cold water fish gelatin (Sigma G-7765) and 0.5% BSA in PBS) for 20' at room temperature. Incubate in primary antibody (diluted in PBG) for 1-2 hours at RT or overnight at 4°C. Wash the slides three times in PBG for 5' each. Incubate with secondary antibody (diluted in PBG) for 1 hour at RT in the dark. Wash the slides three times in PBG for 5' each. Mount in DAPI-containing medium.

Purity and Specificity: This antiserum primarily detects hTERT, but several non-specific bands appear on immunoblots (Figure 1). In immunofluorescence microscopy assays, staining with anti-TERT-16 was specific to the nuclei of cells with ectopic TERT expression.

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to a region near the carboxy terminal end of hTERT (accession number AF018167).

USDA Certification: All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

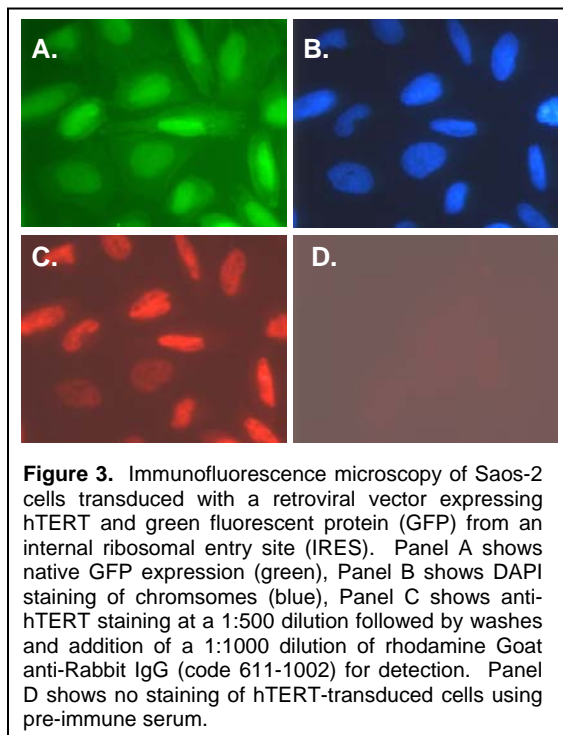


Figure 3. Immunofluorescence microscopy of Saos-2 cells transduced with a retroviral vector expressing hTERT and green fluorescent protein (GFP) from an internal ribosomal entry site (IRES). Panel A shows native GFP expression (green), Panel B shows DAPI staining of chromosomes (blue), Panel C shows anti-hTERT staining at a 1:500 dilution followed by washes and addition of a 1:1000 dilution of rhodamine Goat anti-Rabbit IgG (code 611-1002) for detection. Panel D shows no staining of hTERT-transduced cells using pre-immune serum.

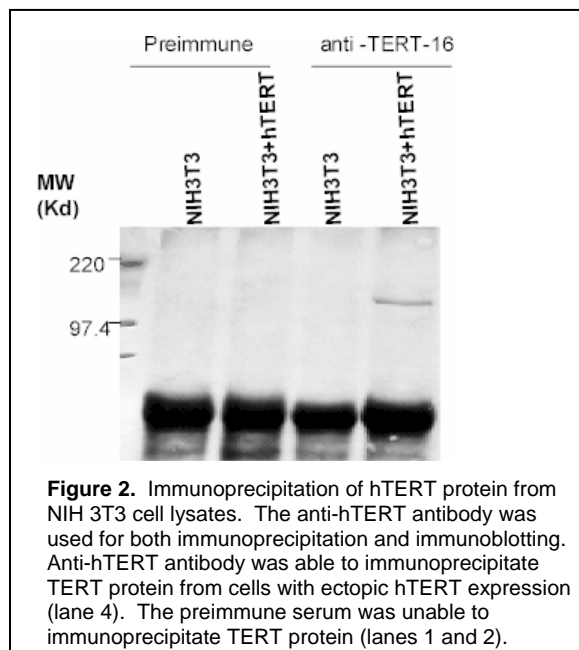


Figure 2. Immunoprecipitation of hTERT protein from NIH 3T3 cell lysates. The anti-hTERT antibody was used for both immunoprecipitation and immunoblotting. Anti-hTERT antibody was able to immunoprecipitate TERT protein from cells with ectopic hTERT expression (lane 4). The preimmune serum was unable to immunoprecipitate TERT protein (lanes 1 and 2).

Specific Reference(s):

Wu, Y.L., *et al.* (2006) Immunodetection of human telomerase reverse-transcriptase (hTERT) re-appraised: nucleolin and telomerase cross paths. *J. Cell Sci.* **119**: 2797-2806.

General Reference(s):

Drissi, R., Zindy, F., Roussel, M. F. and Cleveland, J.L. (2001) c-MYC-mediated regulation of telomerase activity is disabled in immortalized cells. *J. Biol. Chem.* **276**(32): 29994-30001.

Shay, J.W., Zou, Y., Hiyama, E. and Wright, W.E. (2001) Telomerase and cancer. *Hum. Mol. Genet.* **10**: 677-685.

Hiyama, E., Hiyama, K., Yokoyama, T. and Shay, J.W. (2001) Immunohistochemical detection of telomerase (hTERT) protein in human cancer tissues and a subset of cells in normal tissues. *Neoplasia* **3**:17-26.

Wick, M., Zubov, D. and Hagen, G. (1999) Genomic organization and promoter characterization of the gene encoding the human telomerase reverse transcriptase (hTERT). *Gene* **232**: 97-106.

Relevant Links:

Complete [Genomic Sequence](#) The Human Telomerase Gene

Telomere Protein [Database](#)

Human telomerase [components](#), telomere proteins, and proteins involved in the repair of telomeric DNA

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.