Anti-Telomerase catalytic subunit (RABBIT) Antibody - 600-401-252S

Code: 600-401-252S **Size:** 25 µL

Product Description: Anti-Telomerase catalytic subunit (RABBIT) Antibody - 600-401-252S

Concentration: 1.0 mg/mL by UV absorbance at 280 nm

PhysicalState: Liquid (sterile filtered)

Label	Unconjugated
Host	Rabbit
Gene Name	TERT
Species Reactivity	Human
Buffer	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Stabilizer	None
Preservative	0.01% (w/v) Sodium Azide
Storage Condition	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Synonyms	rabbit anti-TERT antibody, rabbit anti-Telomerase catalytic subunit antibody, hTERT, Telomerase reverse transcriptase, HEST2, Telomerase-associated protein 2, TP2, EST2, TCS1, TRT
Application Note	Anti-Telomerase catalytic subunit antibody has been tested for use in immunoblotting, immunoprecipitation, and immunofluorescence microscopy. In these assays, the antibody detects ectopically-expressed hTERT and high levels of endogenous hTERT. A SY5Y cell nuclear extract can be used as a positive control. This antibody primarily detects hTERT, but several non-specific bands appear on immunoblots. In immunofluorescence microscopy assays, staining with anti-TERT-16 was specific to the nuclei of cells with ectopic TERT expression. In immunoblot assays, whole cell or nuclear extracts were loaded at a concentration of 100 µg protein per well. A working dilution of 1:500 anti-TERT antibody was used followed by a 1:3,000 dilution of HRP 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. 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 4ºC. 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.
Background	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.
Purity And Specificity	Affinity Purified Anti-hTERT Antibody was prepared from monospecific antiserum by immunoaffinity chromatography using synthetic peptide coupled to agarose beads. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum. 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).
ELISA	1:10,000 - 1:50,000
Western Blot	1:500
Immunohistochemistry	1:500
IF Microscopy	1:500

Expiration

Expiration date is three (3) months from date of opening.

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).

General Reference

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.

Specific Reference

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.

Related Products

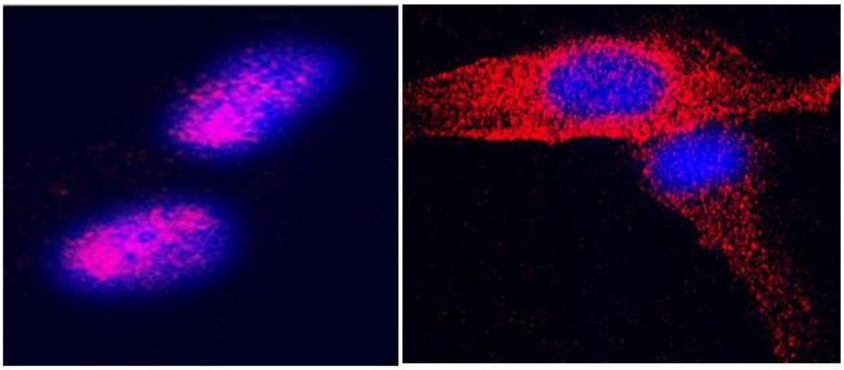
000-000-264	NFKB p65 (Rel A) pS276 CONTROL PEPTIDE - 000-000-264
100-4164	Anti-NFKB p50 (NFKB1) (RABBIT) Antibody - 100-4164
100-4164P	NFKB p50 (NFKB1) CONTROL PEPTIDE - 100-4164P
100-4166	Anti-NFKB cRel (RABBIT) Antibody - 100-4166

Related Links

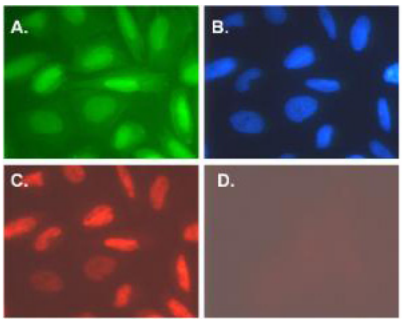
	UniProtKB - O14746
http://www.uniprot.org/uniprot/O14746	
NCBI - O14746.1	http://www.ncbi.nlm.nih.gov/protein/O14746.1
	GeneID - 7015

Images

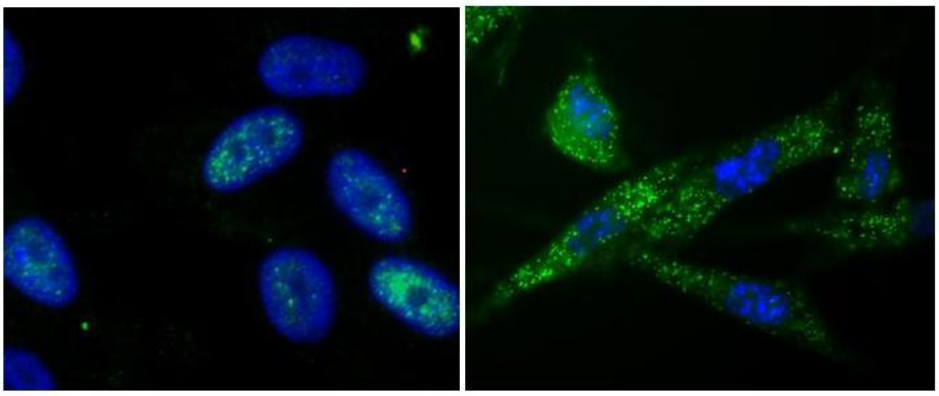
1 Rockland anti-hTERT antibody-Immunofluorescence. Rockland anti-hTERT antibody was used to stain hTERT on hTERT-over-expressing fibroblasts. Cells were untreated (Left) or treated (Right) with 500 uM H202, fixed in 4% PFA (in PBS) for 10 min and frozen in -80 after 3 min air-drying before staining with anti-hTERT 1:2000 overnight. Confocal images provided by G. Saretzki, Institute for Ageing and Health, Newcastle University, UK. See Ahmed et. Al. for more information.



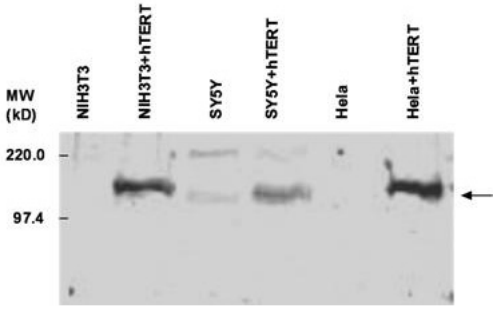
2 Immunofluorescence microscopy of Saos-2 cells transduced with a retroviral vector expressing hTERT and green fluorescent protein (GFP) from an internal ribosomal entry site (IRES). Panel A shows native GFP expression (green), Panel B shows DAPI staining of chromosomes (blue), Panel C shows anti-hTERT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



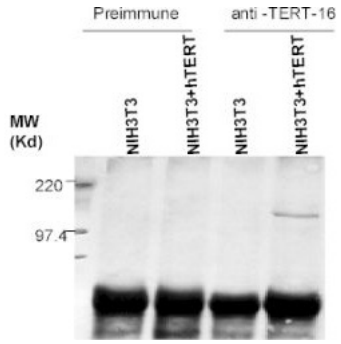
3 Rockland anti-hTERT antibody-Immunofluorescence. Two different lots of anti-hTERT antibody were used to stain hTERT on hTERT-over-expressing fibroblasts. Cells were fixed in 4% PFA (in PBS) for 10 min and frozen in -80 after 3 min air-drying before incubation with Rockland anti hTERT 1:2000 overnight and staining with a 1:2000 dilution of Alexafluor488 secondary Ab. Confocal images provided by G. Saretzki, Institute for Ageing and Health, Newcastle University, UK. See Ahmed et. Al. for more information.



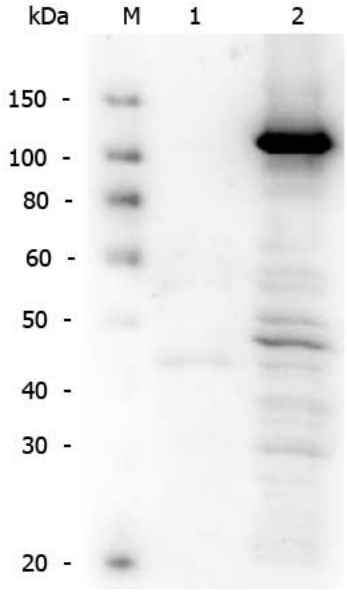
4 Western blot of anti-hTERT antibody.Lane 1: NIH/3T3 cells.Lane 2: NIH/3T3 cells - transduced hTERT expression.Lane 3: SY5Y cells.Lane 4: SY5Y cells - transduced hTERT expression.Lane 5: HeLa cells.Lane 6: HeLa cells - transduced hTERT expression.Primary Antibody: Anti-hTERT at 1:500 dilution.Endogenous levels of mTERT in NIH 3T3 cells (lane 1) and hTERT in HeLa cells (lane 5) are not detectable. The arrow indicates a molecular weight of approximately 127kD, the expected size of hTERT protein.



5 Immunoprecipitation of hTERT protein from NIH 3T3 cell lysates. The anti-hTERT antibody was used for both immunoprecipitation and western blotting. 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).



6 Western Blot of Rabbit anti-Telomerase catalytic subunit antibody. Lane 1: HeLa HV. Lane 2: HeLa 6-2. Load: 10 µg per lane.Primary antibody: Telomerase catalytic subunit antibody at 1:1,000 for overnight at 4°C.Secondary antibody: Peroxidase rabbit secondary antibody (p/n 611-103-122) at 1:40,000 for 45 min at RT.Block: Blocking Buffer for Fluorescent Western Blotting (p/n MB-070).Predicted/Observed size: 127 kDa, 127 kDa for Telomerase catalytic subunit.



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