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Datasheet for 600-401-252S

hTERT Antibody

Overview

Description:	Anti-Telomerase catalytic subunit (RABBIT) Antibody - 600-401-252S
Item No.:	600-401-252S
Size:	25 µL
Applications:	ELISA, IF, IP, WB, ChIP, FISH, IHC, Multiplex
Reactivity:	Human
Host Species:	Rabbit

Product Details

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.
Synonyms:	rabbit anti-TERT antibody, rabbit anti-Telomerase catalytic subunit antibody, hTERT, Telomerase reverse transcriptase, HEST2, Telomerase-associated protein 2, TP2, EST2, TCS1, TRT
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	TERT

Reactivity:	Human
Immunogen Type:	Conjugated Peptide
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).
Purity/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).
Relevant Links:	• 600-401-252 SDS • UniProtKB - O14746 • NCBI - NP_001180305.1 • GeneID - 7015

Application Details

Tested Applications:	ELISA, IF, IP, WB
Suggested Applications:	ChIP, FISH, IHC, Multiplex (Based on references)

Application Note:	Anti-Telomerase catalytic subunit antibody has been tested for use in ELISA, 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.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:50,000
IF:	1:500
IHC:	1:500
IP:	IP 2µL per mg lysate
WB:	1:500

Formulation

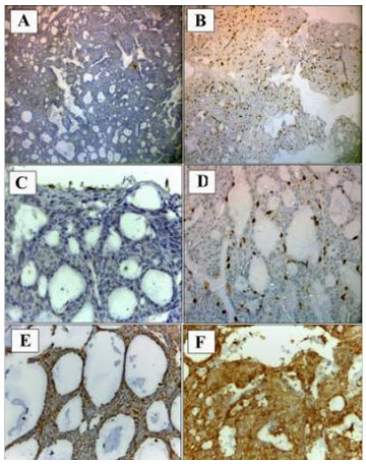
Physical State:	Liquid (sterile filtered)
Concentration:	1.05 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

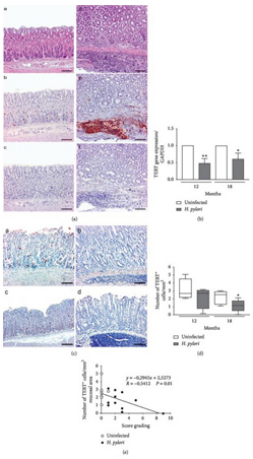
Shipping Condition:	Dry Ice

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.
Expiration:	Expiration date is three (3) months from date of receipt.

Images

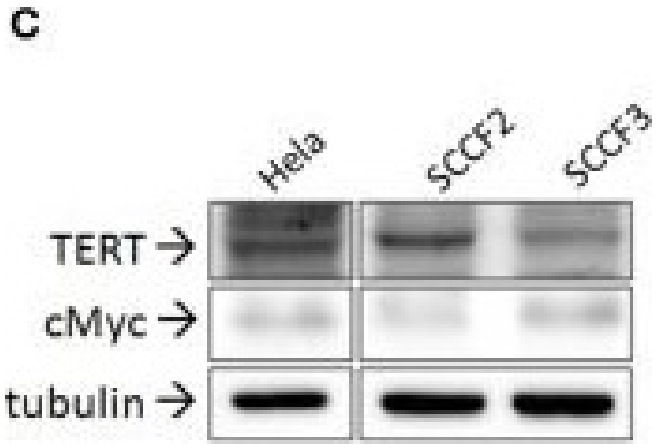


Immunohistochemistry
Immunohistochemistry of proExTMC shows (A) negative expression and (B) positive nuclear expression in HMSC (×100). High magnification (×400) images of (C) negative expression and (D) positive nuclear expression of proExTMC in HMSC. Immunohistochemistry of human telomerase reverse transcriptase (hTERT) shows (E) negative expression and (F) positive cytoplasmic expression in HMSC (×400). Fig 2. PMID: 38584650.



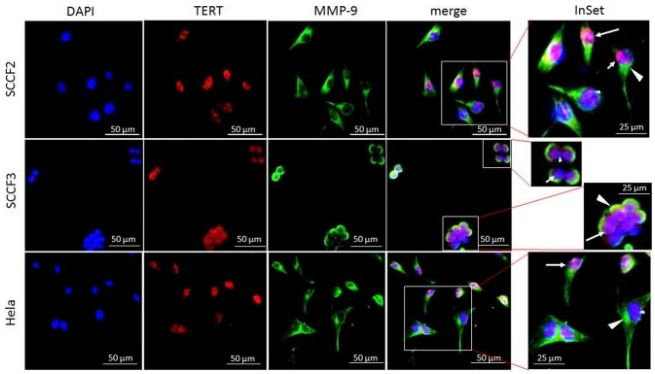
Immunohistochemistry

H. pylori infection decreases mTERT expression in the gastric mucosa of C57BL/6 mice, in the presence of large B lymphocyte aggregates. (a, d) H&E staining and immunostaining of B (b, e) and T (c, f) lymphocytes in gastric sections in infected mice, 12 months after H. pylori SS1 infection (d, e, f) and in control mice (a, b, c). Inflammatory infiltrates were observed in the stomach of infected mice, in the lamina propria and submucosa (c). High number of B lymphocytes (e) and a low number of T lymphocytes (f) were present in the inflammatory cell infiltrates in the infected gastric submucosa (e) compared to uninfected (b and c, respectively). Sections of the stomach from the uninfected mice were negative for both B (b) and T (c) lymphocyte staining. Original magnification ×4, bar: 250 µm (a, b, c), and ×10, bar: 100 µm (d, e, f). (b) mTERT gene expression in gastric tissues of H. pylori SS1-infected mice at 12 and 18 months after infection quantified by real-time qPCR (Taqman). Results are expressed as means ± SD of three independent experiments (infected versus uninfected □p < 0.05; □□p < 0.01). (c) TERT immunolabeling in gastric tissue sections from uninfected mice (a, c) and H. pylori SS1-infected (b, d) after 12 (a, b) and 18 (c, d) months. Lower TERT staining is observed in the gastric mucosa in the area of the inflammatory B lymphocyte infiltrates in infected samples. Original magnification: ×10, bar: 100 µm (a, b), and ×4, bar: 250 µm (c, d). (d) Number of TERT-positive cells/mm2 mucosal area in gastric tissue sections of uninfected and infected samples at 12 and 18 months. Results are expressed as mean ± SD (infected versus uninfected □p < 0.05) according to Mann–Whitney analysis. (e) Inverse correlation between the number of TERT-positive cells/mm2 mucosal area and the total score grading inflammatory lesions in uninfected (white symbols) and infected mice (black symbol), indicating that TERT level decreases with the exacerbation of gastric inflammation. Each symbol represents one mouse. Fig 3. PMID: 32082377

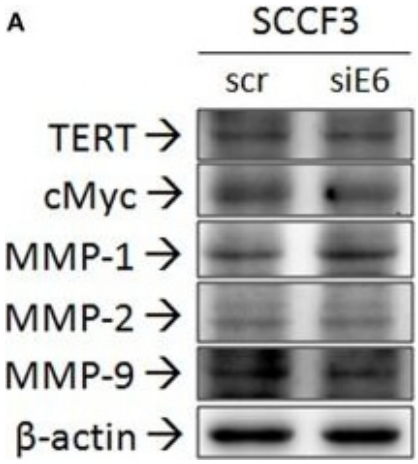


Western Blot

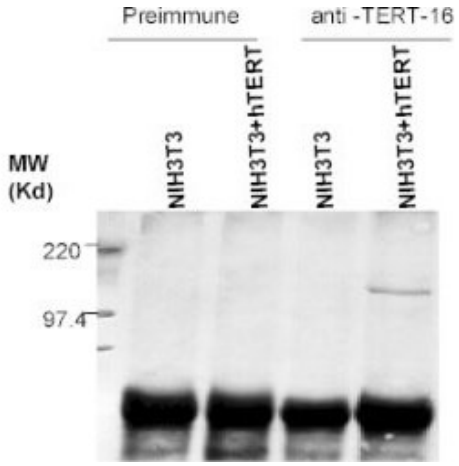
Expression of TERT, cMyc, and telomerase activity in SCCF2 and SCCF3 (A,B) TERT and cMyc gene expression in SCCF2 and SCCF3. Data are normalized for β2-microglobulin expression as housekeeping gene and expressed as relative quantization by the 2−ΔΔCt method. Relative mRNA levels of TERT were lower while cMyc gene expression was higher in SCCF3 with respect to SCCF2 in at least three repeated, independent experiments. (C) A representative Western blotting (WB) gel showing lower TERT protein levels but higher cMyc amounts in SCCF3 vs. SCCF2 is illustrated. Hela whole cell lysate run along with feline samples confirmed the identity of the band. The blot was stripped and reprobed for tubulin to ensure comparable protein loading and allow normalization. Boxes are cut from the same gel at the same exposure time and properly aligned according to molecular standards loaded onto the gel. Full scans from original gels are shown in Supplementary Figure 2. (D,E) Mean densitometric values ± SD from at least three repeated, independent WB experiments, normalized for tubulin expression (statistically significant, *P < 0.05 and **P < 0.01). (F) Telomerase activity was detected in SCCF2 and SCCF3 by telomeric repeat amplification protocol (TRAP) assay. Hela cell lysate was run along with feline samples as positive control. A representative gel out of four independent experiments, showing lower telomerase activity in SCCF3 vs. SCCF2 is illustrated (C–: negative control, sample with no lysate; L100bp: 100 base pairs DNA ladder, the first band from the bottom is 100 bp). (G) Quantification of telomerase activity in SCCF2 and SCCF3 by densitometric analysis expressed in % relative to SCCF2. Data were calculated as the ratio between TERT products ladder and 36 bp internal standard and represent the mean ± standard deviations (SD) of four repeated, independent experiments (statistically significant, **P < 0.01). Figure provided by CiteAb. Source: Front Vet Sci, PMID: 32292795.



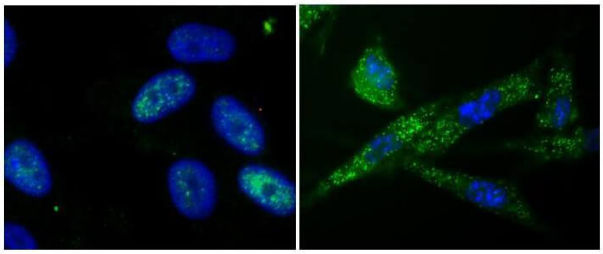
Immunocytochemistry
Sub-cellular localization of TERT and MMP-9 in SCCF2 and SCCF3. Cells were grown on coverslips and subjected to double indirect IF staining for TERT (red fluorescence) and MMP-9 (green fluorescence). Nuclei were counterstained with DAPI. Inset shows higher magnification of merge panel. TERT was localized in the whole nuclear area (long arrow), compartmentalized in proximity of nuclear membrane (short arrow) or in dot-like spots (small arrowhead). MMP-9 was expressed in the cytoplasm (large arrowhead). Hela were stained to ensure antibody reactivity. Figure provided by CiteAb. Source: Front Vet Sci, PMID: 32292795.



Western Blot
Effects of FcaPV-2 E6 gene silencing on TERT, cMyc, and MMP expression in SCCF3 cell line. (A) Representative WB panels showing no changes in TERT, cMyc, MMP-1, MMP-2, and MMP-9 protein levels upon FcaPV-2 E6 gene knock-down siRNA (siE6) vs. scramble RNA (scr) treatment. The blot was stripped and reprobed for β-actin to ensure comparable protein loading and allow normalization. (B) Mean densitometric values normalized for β-actin expression ± standard deviations out of two independent experiments. Figure provided by CiteAb. Source: Front Vet Sci, PMID: 32292795.

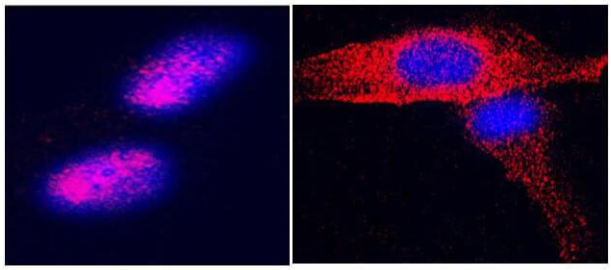


Western Blot
Immunoprecipitation of hTERT protein from NIH 3T3 cell lysates. The anti-hTERT antibody was used for both immunoprecipitation and western blotting. Anti-hTERT antibody was able to immunoprecipitate TERT protein from cells with ectopic hTERT expression (lane 4). The preimmune serum was unable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



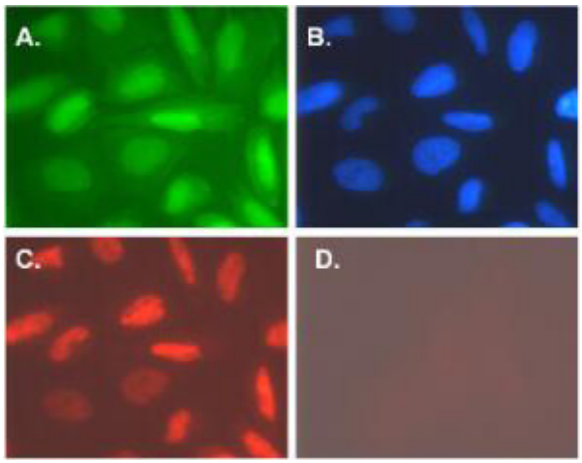
lot 21422, 1:2000 overnight lot 25694, 1:2000 overnight

Immunofluorescence Microscopy
Immunofluorescence of Rockland anti-hTERT antibody - Two different lots of Rockland 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. PMID: 18334557 for more information.

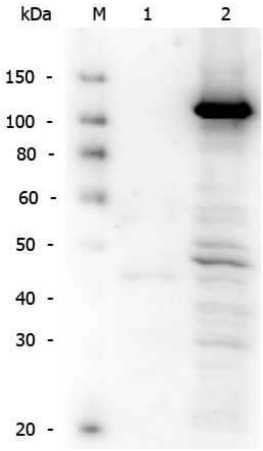


untreated 500 mM H2O2

Immunofluorescence Microscopy
Immunofluorescence of 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 Rockland anti-hTERT 1:2000 overnight. Confocal images provided by G. Saretzki, Institute for Ageing and Health, Newcastle University, UK. PMID: 18334557 for more information.

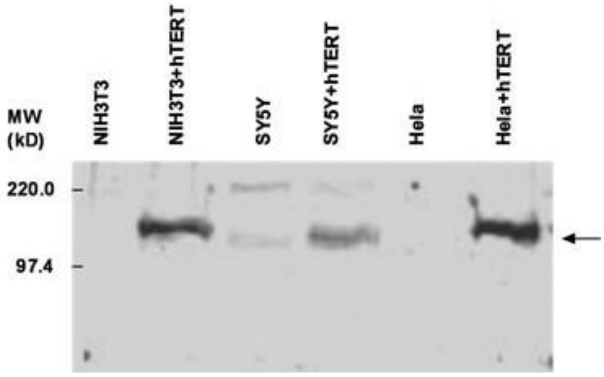


Immunofluorescence Microscopy
Immunofluorescence microscopy of Saos-2 cells transduced with a retroviral vector expressing hTERT and green fluorescent protein (GFP) from an internal ribosomal entry site (IRES). Panel A shows native GFP expression (green), Panel B shows DAPI staining of chromsomes (blue), 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.



Western Blot

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.



Western Blot

Western blot using anti-hTERT antibody (1:500 dilution). Endogenous levels of mTERT in NIH 3T3 cells (lane 1) and hTERT in HeLa cells (lane 5) are not detectable. After transduction with an hTERT expression vector, both cell types show high levels of hTERT protein (lanes 2 and 6). SY5Y cells, which have high endogenous levels of hTERT, have detectable hTERT protein in both untransduced (lane 3) and transduced (lane 4) cells. The arrow indicates a molecular weight of approximately 127kD, the expected size of hTERT protein.

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