Product Datasheet

TERT Antibody
NB100-317SS

Unit Size: 0.025 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

Publications: 11

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NB100-317SS

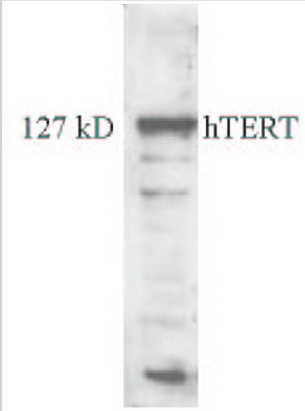
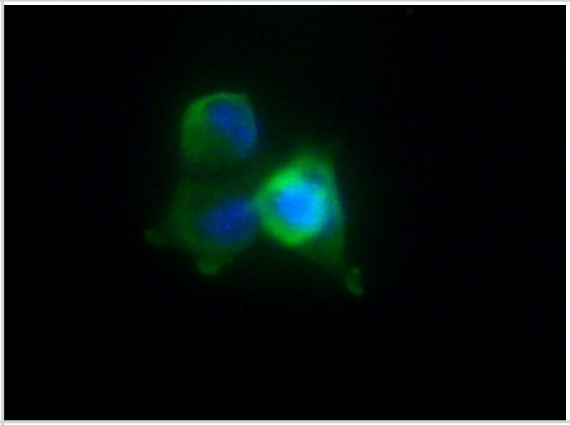
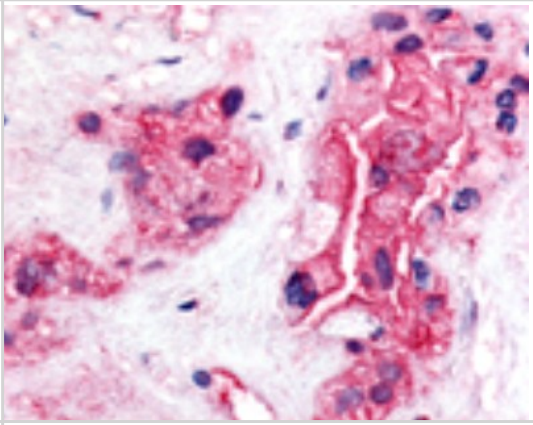
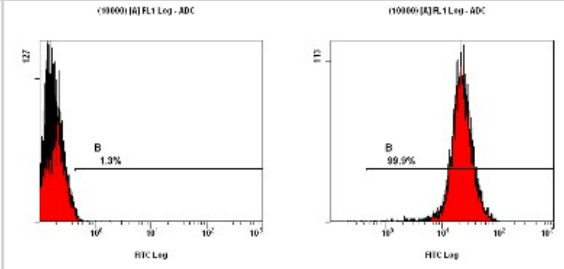
TERT Antibody (2C4)

Product Information	
Unit Size	0.025 ml
Concentration	This product is unpurified. The exact concentration of antibody is not quantifiable.
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	2C4
Preservative	0.02% Sodium Azide
Isotype	IgM
Purity	Ascites
Target Molecular Weight	127 kDa

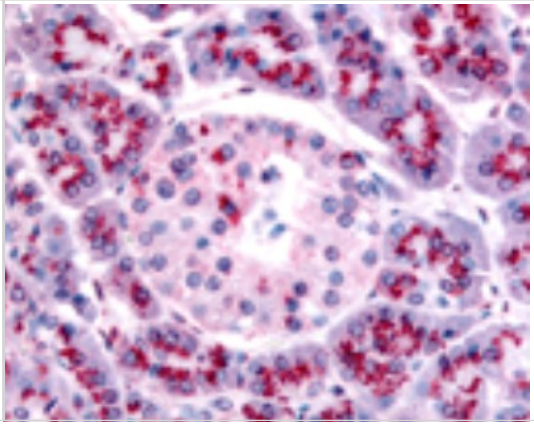
Product Description	
Host	Mouse
Gene ID	7015
Gene Symbol	TERT
Species	Human, Mouse
Species Reactivity	Human and mouse.
Marker	Embryonic Stem Cell Marker
Immunogen	Full-length recombinant human Telomerase reverse transcriptase from insect cells. [UniProt# O14746].
Notes	Licensed to Novus Biologicals LLC under U.S. Patent No 7,285,639.

Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Flow Cytometry 1:50-1:200, Immunocytochemistry/Immunofluorescence 1:50-1:200, Immunohistochemistry 1:50, Immunohistochemistry-Paraffin 1:50, Immunoprecipitation 1:10-1:500, Western Blot 1:500
Application Notes	This Telomerase reverse transcriptase (2C4) antibody is useful for Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry on paraffin-embedded sections, Immunoprecipitation and Western blot. By WB, this antibody recognizes a band at ~127 kDa. An additional background band may be seen at ~110 kDa. Note that the isotype is IgM and the appropriate secondary should be used.

Images

Western Blot: Telomerase reverse transcriptase Antibody (2C4) [NB100-317] - 1:500 dilution on MJ90 cells.	
Immunocytochemistry/Immunofluorescence: Telomerase reverse transcriptase Antibody (2C4) [NB100-317] - Detection of Tert (Green) in HepG2 cells using NB100-317. Nuclei (Blue) are counterstained with Hoechst 33258.	
Immunohistochemistry: Telomerase reverse transcriptase Antibody (2C4) [NB100-317] - Pancreatic carcinoma showing strong staining of tumor cells.	
Flow Cytometry: Telomerase reverse transcriptase Antibody (2C4) [NB100-317] - Staining of NTERA-2 cells using NB100-317at a 1:200 dilution detected using Dylight-488 conjugated goat anti-mouse IgM secondary antibody.	

Immunohistochemistry: Telomerase reverse transcriptase Antibody (2C4) [NB100-317] - Normal pancreas showing moderate staining of exocrine cells and a subset of islets of Langerhans.



Publications

Lotfi RA, El Zawahry KM, Kamar ZA, Hashem Z. Effects of smoking on human telomerase reverse transcriptase expression in the skin. Int. J. Dermatol. 3/6/2014 [PMID: 24601896] (IHC-P, Human)

Fabricius EM, Kruse-Boitschenko U, Khoury R et al. Localization of telomerase hTERT protein in frozen sections of basal cell carcinomas (BCC) and tumor margin tissues. Int J Oncol 2009 Dec [PMID: 19885561] (IHC, Human)

Fabricius EM, Kruse-Boitschenko U, Khoury R et al. Immunohistochemical determination of the appropriate anti-hTERT antibodies for in situ detection of telomerase activity in frozen sections of head and neck squamous cell carcinomas and tumor margin tissues. Int J Oncol 2009 May [PMID: 19360339] (IHC, Human)

Dosset M, Godet Y, Vauchy C et al. Universal cancer peptide-based therapeutic vaccine breaks tolerance against telomerase and eradicates established tumor Clin Cancer Res 2012 Oct 2 [PMID: 23032748] (WB, Mouse)

Masutomi, K et al. The telomerase reverse transcriptase regulates chromatin state DNA damage responses. PNAS 102(23): 8222-8227. 2005 [PMID: 15928077] (WB, Human)

Masutomi K et al. Telomerase maintains telomere structure in normal human cells. Cell. 114(2): 241-53. 2003 [PMID: 12887925] (WB, IP, , Human)

Kim MP, Chen Y, Bekele BN, Lopez A, Khanna A, Chen JQ, Spitz MR, Behrens C, Solis L, Wismach M, Ji L, Wistuba II, Roth JA, Katz RL. Activating enhancer-binding protein-2? nucleolar localization predicts poor survival after stage I non-small cell lung cancer resection. Ann Thorac Surg;92(3):1044-50. 2011 Sep. [PMID: 21871297] (IHC, Human)

Takigami I, Ohno T, Kitade Y, Hara A, Nagano A, Kawai G, Saitou M, Matsuhashi A, Yamada K, Shimizu K. Synthetic siRNA targeting the breakpoint of EWS/Fli-1 inhibits growth of Ewing sarcoma xenografts in a mouse model. Int J Cancer;128(1):216-26. doi: 10.1002/ijc.25564. 2011 Jan 1. [PMID: 20648560] (WB)

Song LL, Ponomareva L, Shen H et al. Interferon-inducible IFI16, a negative regulator of cell growth, down-regulates expression of human telomerase reverse transcriptase (hTERT) gene. PLoS One;5(1):e8569. 2010 Jan 5. [PMID: 20052289]

Khurts S, Masutomi K, Delgermaa L, Arai K, Oishi N, Mizuno H, Hayashi N, Hahn WC, Murakami S. Nucleolin interacts with telomerase. J Biol Chem;279(49):51508-15. 2004 Dec 3. [PMID: 15371412]

Zhang Q, Shi S, Liu Y et al. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol;183(12):7787-98. 2009 Dec 15. [PMID: 19923445]

Procedures

Western Blot protocol for Telomerase reverse transcriptase Antibody (NB100-317)
Western Blot Procedure

1. Resolve protein samples on a 7.5% SDS-PAGE.
2. Transfer proteins to PVDF membranes.
3. Block the membrane with 5% NFDM in PBST overnight at 4C.
4. Dilute primary TERT antibody (NB 100-317) in PBST + 1% BSA.
5. Incubate membrane for 1 hour at RT.
6. Wash 3 times ten minutes on a shaker.
7. Incubate membrane with HRP conjugated secondary for 1 hour (RT), diluted in PBST + 1% BSA.
8. Wash 3 times ten minutes on a shaker.
9. Add ECL reagent, as per kit directions, and expose.

**NOTE: This primary antibody is made in mouse and the isotype of the antibody is IgM.

Immunoprecipitation protocol for Telomerase reverse transcriptase Antibody (NB100-317)

Immobilization of Anti-hTERT anibody
All reagents were from the Seize Primary Mammalian IP Kit.
50 ml of mouse ascites (3.3 mg/ml) was diluted with 350 ml of coupling buffer and coupled to 400 ml of AminoLink Plus slurry per the manufactures instructions. Greater than 80% of the protein in the antibody solution were coupled to the beads.

Immunoprecipitation

1. hTERT was synthesized in rabbit reticulocytes using a pET vector and [35S]-methionine was used to allow visualization of the protein.
2. Beads were washed 2X with wash buffer (WB1): 20 mM Tris-acetate, pH 7.5, 10% glycerol, 1 mM EDTA, 5 mM MgCl2, 100 mM potassium glutamate, 0.1% IGEPAL, and 1 mM DTT, then blocked twice with 250 mL of blocking buffer (20 mM Tris-acetate, pH 7.5, 10% glycerol, 1 mM EDTA, 5 mM MgCl2, 100 mM potassium glutamate, 0.1% IGEPAL, 1 mM DTT, 0.5 mg/mL lysozyme, 0.5 mg/mL BSA, 0.05 mg/mL glycogen, and 0.1 mg/mL yeast RNA) for 15 min at 4C.
3. In between each washing and blocking step the beads were precipitated by centrifugation at 1500g and the supernatant was removed.
4. 50 mL of blocking buffer was then mixed with the 50 mL RNA/protein sample and centrifuged at 17 000g for 10 min at 4C to remove any precipitates.
5. This supernatant was then added to the blocked beads and the samples were mixed on a rotary platform for 2 h at 4C.
6. Following mixing, the beads were washed three times with 325 mL of Wash Buffer #2 (20 mM Tris-acetate, pH 7.5, 10% glycerol, 1 mM EDTA, 5 mM MgCl2, 300 mM potassium glutamate, 0.1% IGEPAL, and 1 mM DTT) and twice with 325 mL of TMG (10 mM Tris-acetate, pH 7.5, 1 mM MgCl2, and 10% glycerol).
7. The beads were precipitated by centrifugation at 1500g in between each wash and the supernatant was removed.
8. The beads were then resuspended in 1X SDS gel loading buffer containing 10 mM DTT and analyzed by SDS PAGE.
9. The immunoprecipitation was also performed on 1x10(7) A549 cells.
10. The beads were assayed by TRAP assay.

Results: IP of [35S]-labled hTERT resulted in 10% yield. This is the same efficiency we observed for anti-HA beads used to IP HA tagged hTERT. IP of telomerase from cells allowed isolation of beads that contained telomerase activity.

Conclusion: We successfully immobilized anti-hTERT antibodies on AminoLink beads using the Seize kit from Pierce. These can be used to immunopurify telomerase. The efficiency should be optimized, but the preliminary results are promising.

Protocol courtesy of Pamela K. Dominick and Michael B. Jarstfer from University of North Carolina, Chapel Hill.

Immunocytochemistry/Immunofluorescence Protocol for Telomerase reverse transcriptase Antibody (NB100-317)
Immunofluorescence

1. Cell growth and feeding for IF
A. Seed cells in 4-chamber slides at 20,000 per chamber.
B. Grow to medium confluence
C. Feed with MCDB170+IP at -48 and -24 hr.

2. Fixing cells for IF
A. Wash cells (~70-80% confluent) with 1XPBS
B. Fix slides each in 1:1 ice cold MEOH:acetone and place at -20C for 10 minutes.
C. Store no more than 48 hr in 100% ethanol.

3. IF for hTERT
A. Remove fixative/ethanol from slides.
B. Add 1 ml 2N HCl to each chamber.
C. Incubate for 20 minutes.
D. Remove the HCl and neutralize with 1 ml 0.1 M Na-borate.
E. Incubate for 5 minutes.
F. Remove Na-borate and add 1 ml blocking buffer.
G. Incubate for 2 hr at RT.
H. Prepare NB 100-297 at indicated dilution.
I. Incubate ON at 4C.
J. Wash 4X5 min. in RT PBS.
K. Add secondary (FITC conjugated rabbit anti-mouse IgM).
L. Incubate at RT for 2 hrs.
M. Wash 4X5 min. in 1X PBS.
N. Wash 5 min in 1X PBS with DAPI (1.5 ug/ml).
O. Rinse slides briefly on PBS.
P. Remove chambers from slides.
Q. Mount in Vectashield (Vector catalog # H1200) and observe.

Blocking buffer To 500 ml of 1X PBS:
A. 5 g fish gelatin (Sigma catalog #G7765)
B. 25 ml goat serum
C. 5 g BSA Filter through 0.2 u filter and store at 4C

Immunohistochemistry - FFPE sections Protocol specific for Telomerase reverse transcriptase Antibody (NB100-317)
Immunohistochemistry - FFPE sections

I. Deparaffinization:
A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:

To Prepare 200 ml of Quenching Solution: Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.
**Use within 4 hours of preparation

A. Place slides in peroxidase quenching solution: 15-30 minutes.
B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:
A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96C.

B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.
E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:
A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen.
B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of primary antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
I. Wash slides with Wash Solution: 3 changes for 5 minutes each
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.
N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:
- Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
- Prior to deparaffinization, heat slides overnight in a 60 degrees celcius oven.
- All steps in which Xylene is used should be performed in a fume hood.
- For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
- For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
- 200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. for small tissue sections less than 200 ul may be used.
- 5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.
- Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1.5 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).

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Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our guarantee, please visit www.novusbio.com/guarantee.