Product Datasheet

TdT Antibody - BSA Free

NBP2-31368

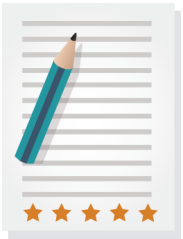
Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

www.novusbio.com technical@novusbio.com

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NBP2-31368

Updated 2/23/2025 v.20.1



NBP2-31368

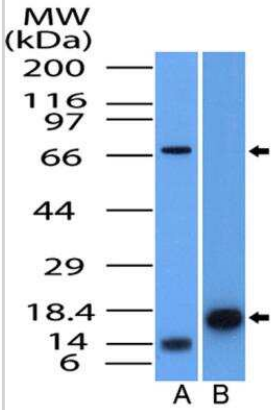
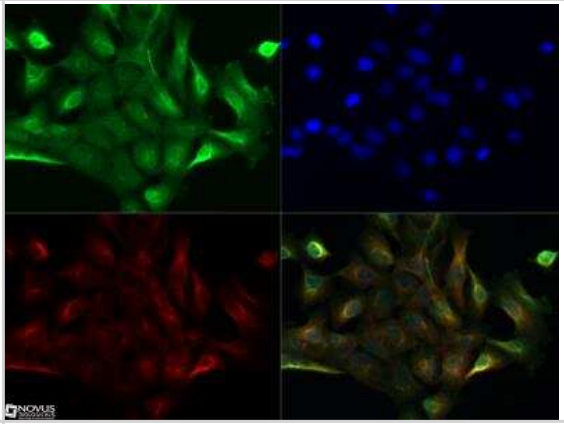
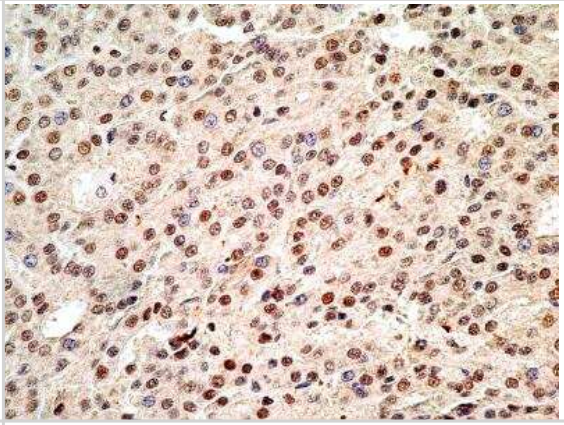
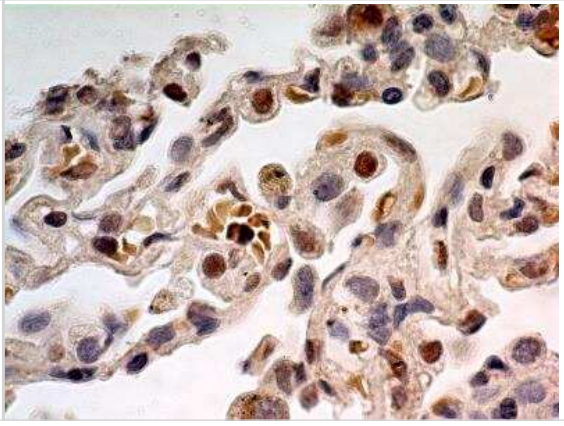
TdT Antibody - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Protein A purified
Buffer	PBS
Target Molecular Weight	58.5 kDa

Product Description	
Host	Rabbit
Gene ID	1791
Gene Symbol	DNTT
Species	Human
Reactivity Notes	Immunogen's sequence similarity with other species: Rat (77%) Mouse (74%), Bovine (78%)
Immunogen	Partial recombinant protein made to an internal sequence of human TdT (between residues 50-300) [UniProt# P04053]

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1.0 ug/ml, Immunohistochemistry 10 ug/ml, Immunocytochemistry/ Immunofluorescence 1:50 - 1:100, Immunohistochemistry-Paraffin 10 ug/ml
Application Notes	TdT is a 509 amino acids long protein with predicted molecular weight of 58.5 kDa, and it localizes mainly to the nucleus with low amounts in cytoplasm of the cells. In our Western blot validation with MOLT4 lysate, this TdT antibody detected a single specific band at ~66 kDa position. In our immunohistochemistry validation on formalin fixed paraffin embedded tissue sections, this antibody developed a strong nuclear/ weak cytoplasmic staining in normal lung and hepatocellular carcinoma of human. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Images

Western Blot: TdT Antibody [NBP2-31368] - WB detection of TDT protein in (A) lysate of MOLT4 human leukemia cell line and (B) partial recombinant protein by using TDT antibody. Primary antibody concentration used: 1 ug/ml for Molt4 lysate, 0.05 ug/ml for recombinant protein.	
Immunocytochemistry/Immunofluorescence: TdT Antibody [NBP2-31368] - TdT antibody was tested in A431 cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red). An antibody concentration of 0.01 ug/ml was used. Image objective 40x.	
Immunohistochemistry-Paraffin: TdT Antibody [NBP2-31368] - IHC-P analysis of TdT protein in a section of human hepatocellular carcinoma (HCC) using TdT antibody at a concentration of 10 ug/ml. The HCC cells depicted strong nuclear / weak cytoplasmic positivity for TdT immunostaining.	
Immunohistochemistry-Paraffin: TdT Antibody [NBP2-31368] - IHC-P analysis of TdT protein in a section of human lung using TdT antibody at a concentration of 10 ug/ml. The alveolar cells of the lungs showed strong nuclear / weak cytoplasmic positivity for TdT immunostaining.	

Procedures

Western Blot protocol for TdT Antibody (NBP2-31368)
TdT Antibody:
Reagents needed:
a. Washing Buffer: Tris Buffer Saline with 0.01% of tween 20).
b. Blocking Buffer: 5% skimmed milk powder in washing buffer).
c. Secondary antibody, Horseradish peroxidase conjugated.
d. Chemiluminescent solution (SuperSignal WestPicoTM, Pierce).

Western blot Method:
1. Perform SDS-PAGE using PVDF membrane. Cut into strips.
2. Activate strips with methanol by dipping them into methanol for 5 min.
3. Discard the methanol and take fresh methanol to repeat step b.
4. Let the strips dry, and then add blocking solution and incubate at RT in a shaker for 30-45 minutes.
5. Dilute primary antibody in blocking buffer. Incubate the number of strips required with the diluted primary antibody at room temperature for 2 hours in a shaker.
6. Wash strips two times with washing buffer at 30 minutes intervals.
7. Dilute HRP conjugated secondary antibody in blocking buffer. Add diluted secondary antibody to the membrane strips and incubate for exactly 1 hour while shaking at RT.
8. Wash the strips with washing buffer for 2-3 hours with 3 to 4 changes on a shaker. This helps in reducing the back ground staining.
9. Prepare the chemiluminescent solution (SuperSignal WestPicoTM) by mixing solution A and Solution B at 1:1. Mix well. Soak the strip in the chemiluminescent solution; keep for 3-5 minutes under constant shaking.
10. Expose the membrane to a sheet of film and develop.

Immunocytochemistry/Immunofluorescence protocol for TdT Antibody (NBP2-31368)
TdT Antibody:
Immunocytochemistry Protocol

Culture cells to appropriate density on suitable glass coverslips in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 5-10 minutes.
2. Remove the formalin and add 0.5% Triton-X 100 in TBS to permeabilize the cells. Incubate for 5-10 minutes.
3. Remove the permeabilization buffer and add wash buffer (i.e. PBS or PBS with 0.1% Tween-20). Be sure to not let the specimen dry out. Gently wash three times for 10 minutes.
4. Alternatively, cells can be fixed with -20C methanol for 10 min at room temperature. Remove the methanol and rehydrate in PBS for 10 min before proceeding.
5. To block nonspecific antibody binding incubate in 10% normal goat serum for 1 hour at room temperature.
6. Add primary antibody at appropriate dilution and incubate at room temperature for 1 hour or at 4 degrees C overnight.
7. Remove primary antibody and replace with wash buffer. Gently wash three times for 10 minutes.
8. Add secondary antibody at the appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove antibody and replace with wash buffer. Gently wash three times for 10 minutes.
10. Nuclei can be staining with 4',6' diamino phenylindole (DAPI) at 0.1 ug/ml, or coverslips can be directly mounted in media containing DAPI.
11. Cells can now be viewed with a fluorescence microscope.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow proper laboratory procedures for the disposal of formalin.

Immunohistochemistry-Paraffin protocol for TdT Antibody (NBP2-31368)

TdT Antibody:

1. Deparaffinize the tissue sections by immersing the slides in Xylene with two changes for 10 min each. Sections should not get dried at any stage from this point.
2. Rehydrate the tissue sections by immersing the slides in decreasing grades of ethanol as follows:
 a. Immerse in 100% ethanol with 2 changes for 5 minutes each
 b. Immerse in 95% ethanol with 2 changes for 5 minutes each
 c. Immerse in 90% ethanol for 5 minutes
 d. Immerse in 70% ethanol for 5 minutes
 e. Immerse in 50% ethanol for 5 minutes
 f. Immerse in distilled water for 5 minutes
3. Antigen Retrieval (Microwave Method):
 a. Immerse the slides in a microwave compatible tray containing 10 mM Sodium Citrate buffer (pH 6.0) with 0.05% Tween 20.
 b. Boil the slides and maintain the sub-boiling temperature for 5 minutes in the microwave. Thereafter, take out the tray very carefully and cool it at room temperature (RT) for about 30 minutes.
 c. Wash the slides 3 times, 3 minutes each by immersing them in TBST (Tris Buffered Saline having 0.05% Tween 20).
4. Quenching of Endogenous Peroxidase:
 a. Incubate the slides in 3% hydrogen peroxide prepared in methanol for 15 minutes (at RT, in dark conditions).
 b. Wash the slides in TBST 3 times, 3 minutes each.
5. Protein Blocking:
 a. Incubate the sections with background sniper solution at RT for 15 minutes (Biocare Medicals, USA).
 b. Wash the sections 3 times, 3 min each by immersing the slides in TBST.
6. Primary Antibody:
 a. Dilute the primary antibody at 5ug/ml concentration using PBS as a diluent.
 b. Incubate the sections with diluted primary antibody for 90 minutes at RT in a humidified chamber.
 c. Thereafter, wash the slides 4 times, 5 minutes each with TBST.
7. Probe (Secondary Reagent):
 a. Incubate with MACH 1 Mouse probe for 15 minutes at RT.
 b. Incubate for 30 min at room temperature with HRP-Polymer (Biocare Medical, USA).
 c. Wash the slides with TBST 4 times, 5 minutes each
8. Chromogen:
 a. Mix 32ul of DAB Chromogen with 1 ml of DAB substrate buffer (Biocare Medical, USA).
 b. Apply 200ul DAB mixture/section and incubate at RT in dark conditions (few seconds - 5 minutes).
 c. As soon as an appropriate color develops, rinse the slides with deionized water (2-3 brief rinses).
9. Counter stain:
 a. Counter stain with Hematoxylin for 30 seconds (Vector Labs, USA).
 b. Wash in deionized water for 1-2 minutes to clear the extra stain.
 c. Incubate the slides in bluing solution or Scott's water twice for 2 minutes each time.
10. Dehydrate the sections in increasing grades of alcohols:
 a. 50% alcohol for 1 minute
 b. 70% for 1 minute
 c. 90% for 1 minute
 d. 95% for 1 minute
 e. 100% for 1 minute
 f. Xylene with 2 changes for 2 minutes each
11. Mount with DPX mount and cover-slip glass (Fisher Scientific, USA), carefully not allowing any air bubbles to enter.

NOTE:- This protocol is provided as a reference tool only. Depending upon the type of tissues /tissue processing and reagents employed, the end user will need to optimize the final conditions for achieving an expected staining.

Novus Biologicals USA
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

Products Related to NBP2-31368

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NBP3-21261PEP	TdT Recombinant Protein Antigen

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 100% guarantee, please visit www.novusbio.com/guarantee

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NBP2-31368

Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications