Product Datasheet

TLR9 Antibody (2A4C6.2E5) - BSA Free
NBP2-31150

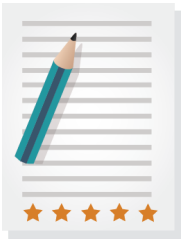
Unit Size: 0.1 mg

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/NBP2-31150

Updated 10/23/2024 v.20.1



NBP2-31150

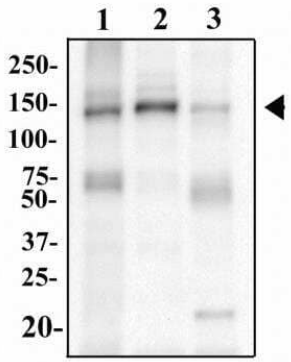
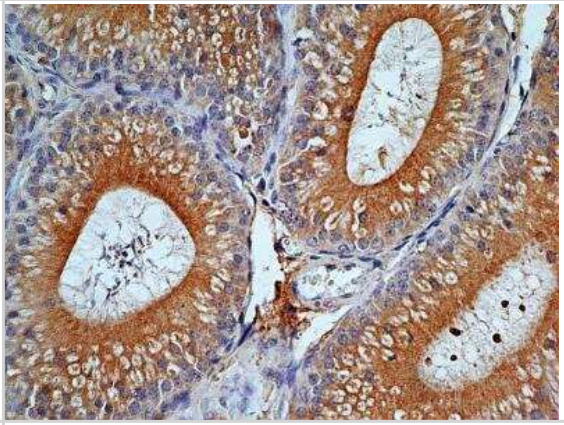
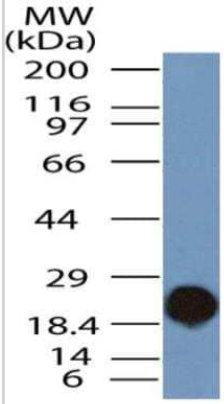
TLR9 Antibody (2A4C6.2E5) - BSA Free

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	Monoclonal
Clone	2A4C6.2E5
Preservative	0.05% Sodium Azide
Isotype	IgG2a Kappa
Purity	Protein G purified
Buffer	PBS

Product Description	
Host	Rat
Gene ID	54106
Gene Symbol	TLR9
Species	Mouse
Immunogen	Partial recombinant protein made to an internal portion of mouse TLR9 (between amino acids 500-800) [UniProt Q9EQU3]

Product Application Details	
Applications	Western Blot, Immunohistochemistry, Immunohistochemistry-Paraffin, ICC/IF (Negative)
Recommended Dilutions	Western Blot 2 ug/mL, Immunohistochemistry 2 ug/mL, Immunohistochemistry-Paraffin 2-5 ug/mL, ICC/IF (Negative)

Images

Western Blot: TLR9 Antibody (2A4C6.2E5) [NBP2-31150] - Mouse Liver (lane 1), Mouse Kidney (lane 2) and Mouse Spleen (lane 3) were separated by SDS-PAGE and the protein transferred to PVDF. The membrane was then probed with anti-TLR9 at 2ug/mL and detected with an anti-rat HRP secondary antibody using chemiluminescence.	
Immunohistochemistry-Paraffin: TLR9 Antibody (2A4C6.2E5) [NBP2-31150] - Analysis of TLR9 protein in normal mouse epididymis section using TLR9 antibody (clone 2E5) at a concentration of 5 ug/mL. This antibody developed strong cytoplasmic staining in the pseudostratified columnar epithelial cells as well as the blood vessels of epididymis. The basal cells, the smooth muscle cells and the mature sperm cells were largely found negative for TLR9 staining.	
Western Blot: TLR9 Antibody (2A4C6.2E5) [NBP2-31150] - TLR9 Antibody (2E5) [NBP2-31150] - TLR9 Antibody (2E5) [NBP2-31150] - WB analysis of partial recombinant TLR9 protein (expected molecular weight ~21kDa) with TLR9 antibody (clone 2A4C6.2E5) at a concentration of 2 ug/mL.	

Procedures

Western Blot protocol for TLR9 Antibody (NBP2-31150)
TLR9 Antibody (2A4C6.2E5):
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 25 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute anti-TLR9 primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.
Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunohistochemistry-Paraffin protocol for TLR9 Antibody (NBP2-31150)

TLR9 Antibody (2A4C6.2E5):

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 NBP2-31150

NBP2-26232	CpG oligodeoxynucleotides with negative control, TLR9 ligand
HAF005	Goat anti-Rat IgG Secondary Antibody [HRP]
NBP1-75398	Goat anti-Rat IgG (H+L) Secondary Antibody (Pre-adsorbed)
NBP1-43321-0.5mg	Rat IgG2a Kappa Light Chain Isotype Control (R2a)

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/NBP2-31150

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