Product Datasheet

HLA DMA Antibody (5C9NB) - BSA Free
NBP2-44300

Unit Size: 0.1 mg

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

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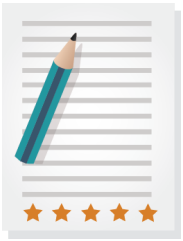


NBP2-44300

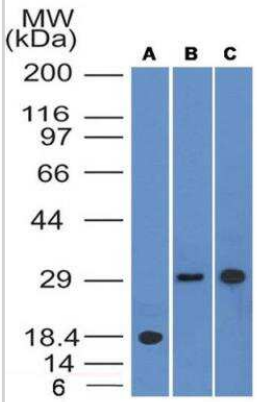
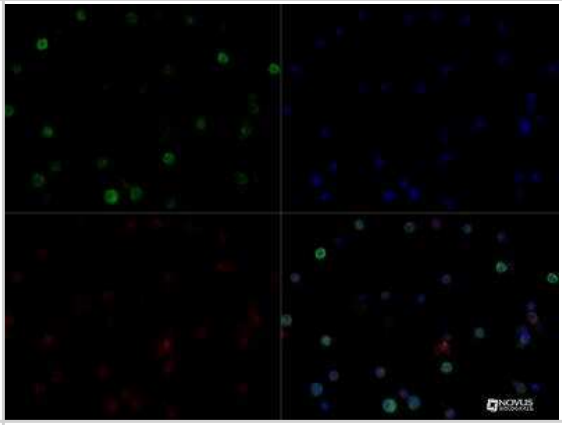
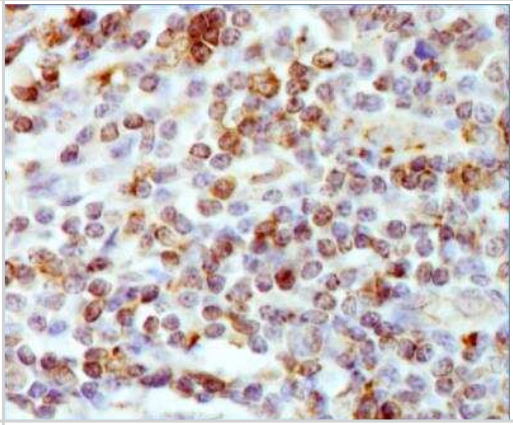
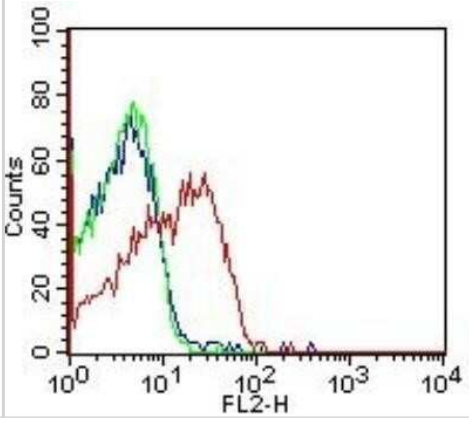
HLA DMA Antibody (5C9NB) - BSA Free

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	5C9NB
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS

Product Description	
Host	Mouse
Gene ID	3108
Gene Symbol	HLA-DMA
Species	Human
Immunogen	Partial recombinant human HLA DMA protein (between amino acids 10-300) [Entrez NP_006111]

Product Application Details	
Applications	Western Blot, Flow (Cell Surface), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:500, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:50 - 1:100, Immunohistochemistry-Paraffin 1:200, Flow (Cell Surface) 2.5 ug / million cells

Images

Western Blot: HLA DMA Antibody (5C9NB) [NBP2-44300] - Western blot analysis of HLA DMA (5C9NB) in A. partial recombinant human HLA DMA protein B. Ramos lysate and C. Raji lysate.	
Immunocytochemistry/Immunofluorescence: HLA DMA Antibody (5C9NB) [NBP2-44300] - Daudi cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with MHC Class II (5C9NB) at a 1:40 dilution for 1 hour at room temperature and detected with Dylight 488 (Green). Actin was detected using Phalloidin 568 (Red). Nuclei were detected using DAPI (Blue). Cells were imaged using a 40X objective.	
Immunohistochemistry: HLA DMA Antibody (5C9NB) [NBP2-44300] - IHC analysis of a formalin fixed and paraffin embedded tissue section of normal human tonsil using 1:200 dilution of HLA DMA antibody (clone 5C9NB). The antibody generated a diffused to punctate staining in cellular membranes and cytoplasm, especially in the perinuclear region representing late endosomal localization of major histocompatibility complex, class II, DM alpha/HLA DMA protein.	
Flow (Cell Surface): HLA DMA Antibody (5C9NB) [NBP2-44300] - Analysis using the Azide Free version of NBP2-44300. Detection of Human PBMC cells (surface) with HLA DMA (5C9NB) antibody (red) or isotype control (mouse IgG1; green). Blue line represents cells alone. Positive staining was observed using PE conjugated mouse anti-IgG(H +L) secondary antibody. Live cells were gated (FL-2) for analysis.	

Procedures

Western Blot protocol for HLA DMA Antibody (NBP2-44300)
HLA DMA Antibody (5C9NB):
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-HLA DMA 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 HLA DMA Antibody (NBP2-44300)

HLA DMA Antibody (5C9NB):

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.

Immunocytochemistry/Immunofluorescence protocol for HLA DMA Antibody (NBP2-44300)
HLA DMA Antibody (5C9NB):
Immunocytochemistry Protocol

Culture cells to appropriate density 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 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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 NBP2-44300

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)
NBP2-44300FR	HLA DMA Antibody (5C9NB) [DyLight 680]

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.

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