Product Datasheet

HLA DMA Antibody (6B12NB) - BSA Free
NBP2-44302

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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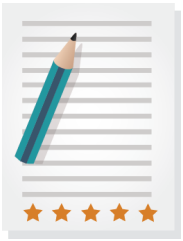


NBP2-44302

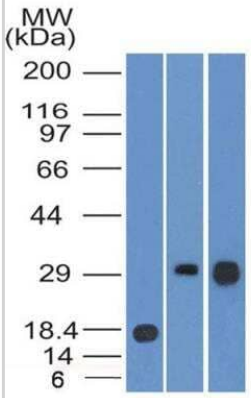
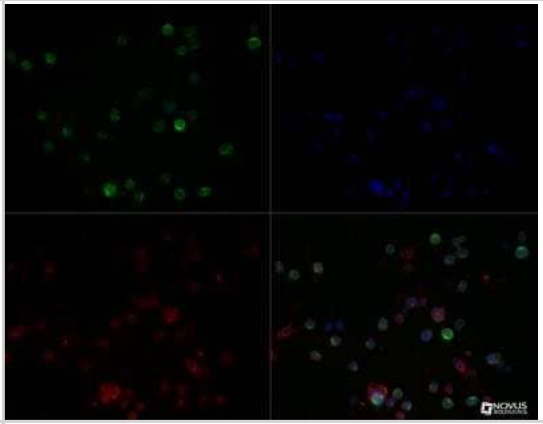
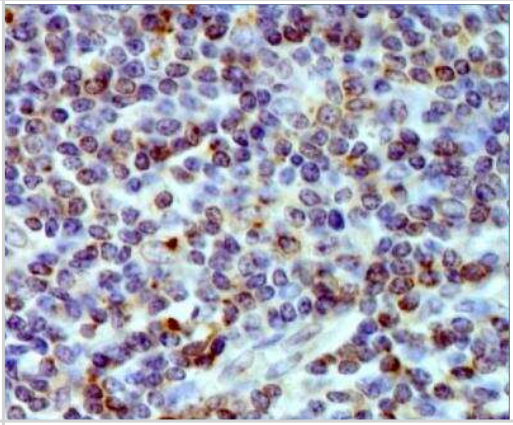
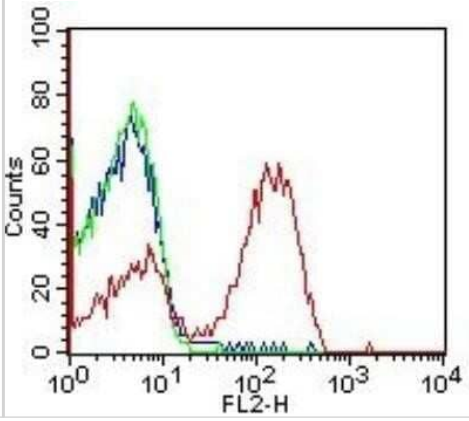
HLA DMA Antibody (6B12NB) - BSA Free

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	6B12NB
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 (6B12NB) [NBP2-44302] - Western blot analysis of (A) partial recombinant human HLA DMA protein, and lysates of (B) Ramos cells and (C) Raji cells using 1:500 dilution of HLA DMA antibody (clone 6B12NB)	
Immunocytochemistry/Immunofluorescence: HLA DMA Antibody (6B12NB) [NBP2-44302] - 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 (6B12NB) 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-Paraffin: HLA DMA Antibody (6B12NB) [NBP2-44302] - IHC analysis of a formalin fixed and paraffin embedded tissue section of normal human tonsil using 1:200 dilution of HLA DMA antibody (clone 6B12NB). The antibody generated a punctate staining in the cytoplasm/perinuclear region representing late endosomal localization of major histocompatibility complex, class II, DM alpha/HLA DMA protein.	
Flow (Cell Surface): HLA DMA Antibody (6B12NB) [NBP2-44302] - Analysis using the Azide Free version of NBP2-44302. Detection of Human PBMC cells (surface) with HLA DMA (6B12NB) 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 (NBP2-44302)
HLA DMA Antibody (6B12NB):
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%.

Immunocytochemistry/Immunofluorescence protocol for HLA DMA Antibody (NBP2-44302)
HLA DMA Antibody (6B12NB):
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.

Immunohistochemistry-Paraffin protocol for HLA DMA Antibody (NBP2-44302)
HLA DMA Antibody (6B12NB):
I. Deparaffinization and rehydration

Prior to staining, tissue sections must be deparaffinized and rehydrated. Incomplete removal of paraffin can cause poor staining of the section.

1. Immerse slides in xylene and incubate for 5 minutes. Repeat twice with fresh xylene for another 5 minutes each.
2. Immerse slides in 100% ethanol for 5 minutes, and follow with immersion in 95%, 75% and 50% ethanol for 3 minutes each.
3. Rinse slides with distilled water for 5 minutes; keep in water until ready to perform antigen retrieval.

II. Heat induced antigen retrieval (HIAR)

Most formalin fixed tissue requires an antigen retrieval step before immunohistochemical staining can proceed. Heat induced antigen retrieval can be performed using a steamer, pressure cooker, or a microwave. The retrieval time written in this protocol is based on using a retrieval steamer. The heating time may need to be adjusted if you use a different device and method.

1. Fill plastic Coplin jar/container with Antigen Retrieval Buffer.
2. Place the Coplin jar/container in steamer.
3. Turn on steamer and preheat to 90-100 degrees C. Carefully put slides into the Coplin jar/container and steam for 40 min (95-100 degrees C).
4. Turn off the steamer, remove the Coplin jar, place at room temperature and allow slides to cool for 20 min.
5. Rinse slide by incubation of slide in distilled water for 3 minutes. Repeat this step twice and begin staining procedure.

III. Staining
1. Wash sections in dH2O three times for 5 minutes each.
2. Wash section in wash buffer (1X PBS/0.1% Tween-20 (1X PBST)) for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1X PBST, 5% goat serum) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul primary antibody diluted in 1X PBST, 5% goat serum to each section. Incubate overnight at 4 degrees C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated secondary antibody, diluted in 1X PBST, 5% goat serum. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in dH2O.
12. Counterstain sections in hematoxylin.
13. Wash sections in dH2O two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Antigen Retrieval Buffer: 0.01M Citrate Buffer, pH6.0.
Prepare Stock Solution:
A. 0.1M Sodium Citrate;
B. 0.1M Citric Acid
Make 250mL 1X antigen retrieval buffer each time before use by mixing 20.5mL stock solution A. with 4.5mL stock solution B. and add distilled water to 250mL.

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Products Related to NBP2-44302

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)
NBP2-44302APC	HLA DMA Antibody (6B12NB) [Allophycocyanin]

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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