

Product Datasheet

CCR2 Antibody (2D8) - BSA Free NBP2-35333

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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NBP2-35333

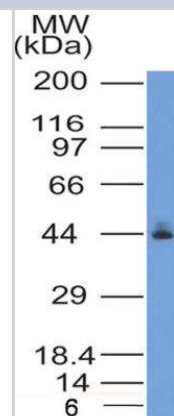
CCR2 Antibody (2D8) - BSA Free

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	2D8
Preservative	0.05% Sodium Azide
Isotype	IgG2b Lambda
Purity	Protein G purified
Buffer	PBS
Product Description	
Description	Novus Biologicals Mouse CCR2 Antibody (2D8) - BSA Free (NBP2-35333) is a monoclonal antibody validated for use in IHC and WB. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	729230
Gene Symbol	CCR2
Species	Human
Immunogen	A synthetic peptide made to an N-terminal portion of the human CCR2 protein (between residues 1-100) [Uniprot: P41597]
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Immunohistochemistry
Recommended Dilutions	Western Blot 2 ug/ml, Immunohistochemistry 5 ug/ml, Immunohistochemistry-Paraffin 5 ug/ml

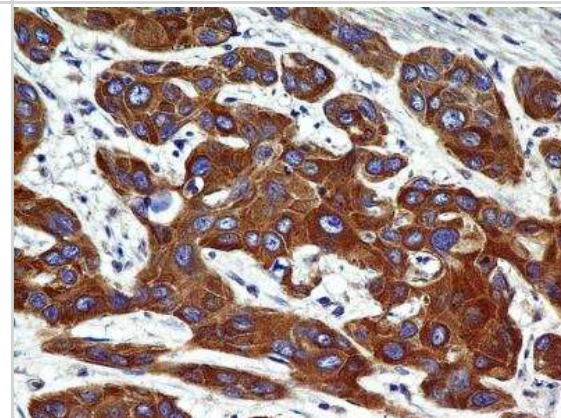


Images

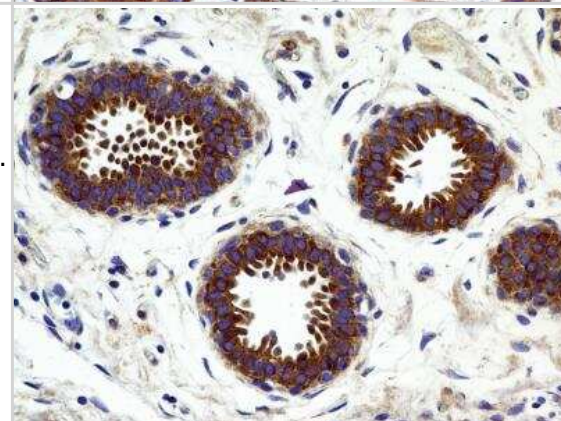
Western Blot: CCR2 Antibody (2D8) [NBP2-35333] - Analysis of CCR2 [2D8] in Molt4 lysate



Immunohistochemistry-Paraffin: CCR2 Antibody (2D8) [NBP2-35333] - Analysis of FFPE tissue section of human esophageal squamous cell carcinoma (SCC) using CCR2 antibody (clone 2D8) at 5 ug/ml concentration. The cancer cells depicted strong cytoplasmic-membranous immunostaining, whereas the cells of tumor stroma were largely negative for CCR2 immunopositivity.



Immunohistochemistry-Paraffin: CCR2 Antibody (2D8) [NBP2-35333] - Analysis of FFPE tissue section of human breast using CCR2 antibody (clone 2D8) at 5 ug/ml concentration. The glandular cells in the lobules showed a specific cytoplasmic-membranous immunostaining, whereas, the inter-lobular connective tissue did not develop any staining for CCR2.



Procedures

Western Blot protocol for CCR2 Antibody (NBP2-35333)

CCR2 Antibody (2D8):

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-CCR2 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 CCR2 Antibody (NBP2-35333)**CCR2 Antibody (2D8):**

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.



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Products Related to NBP2-35333

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-48338PEP	CCR2 Antibody Blocking Peptide
210-TA-005	TNF-alpha [Unconjugated]

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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