Product Datasheet

Flt-3/Flk-2/CD135 Antibody (7E8.2C8) - BSA Free
NBP2-42210

Unit Size: 0.1 mg

Store at -20C. Avoid freeze-thaw cycles.

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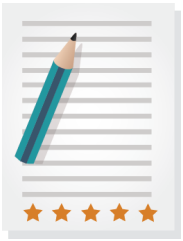
Updated 10/23/2024 v.20.1



NBP2-42210

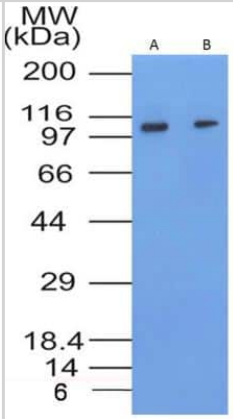
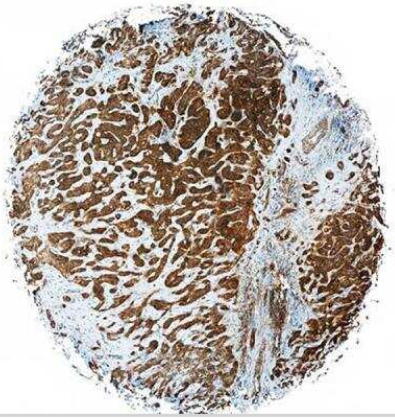
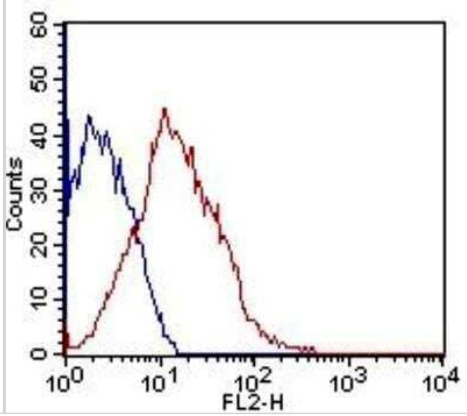
Flt-3/Flk-2/CD135 Antibody (7E8.2C8) - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	7E8.2C8
Preservative	0.02% Sodium Azide
Isotype	IgG2b Lambda
Purity	Protein G purified
Buffer	PBS

Product Description	
Host	Mouse
Gene ID	2322
Gene Symbol	FLT3
Species	Human
Reactivity Notes	Immunogen displays the following percentage of sequence identity for non-tested species: ~86% to Mouse and Rat's FLT3 protein.
Immunogen	Partial recombinant human FLT3 protein (between amino acids 250-500) [UniProt P36888]

Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 2 ug/ml, Flow Cytometry 2 ug/million cells, Immunohistochemistry 5 -15 ug/ml, Immunohistochemistry-Paraffin 5-15 ug/ml
Application Notes	FLT3 is a 993 amino acids long protein with predicted molecular weight of 112.9 kDa, however, it undergoes extensive glycosylation and phosphorylation so that the mature protein will have relatively higher molecular weights. In WB assay, the glycosylated protein species may run between 113-160 kDa position, while the phosphorylated protein may result on generation of a doublet band.

Images

Western Blot: Flt-3/Flk-2/CD135 Antibody (7E8.2C8) [NBP2-42210] - WB analysis of lysates from (A) Human Embryonic Kidney 293 / HEK293 and (B) Human Embryonic carcinoma NCCIT cell lines using Flt-3/Flk-2/CD135 antibody (clone 7E8.2C8) at 2ug/ml concentration. The antibody detected single specific bands in both samples at the expected molecular weight position.	
Immunohistochemistry-Paraffin: Flt-3/Flk-2/CD135 Antibody (7E8.2C8) [NBP2-42210] - IHC analysis of a formalin fixed paraffin embedded tissue section of human liver cancer using Flt-3/Flk-2/CD135 antibody (clone 7E2.2C8) at 15 ug/ml concentration. The antibody generated specific cytoplasmic immunostaining of FLT3 in the cancerous cells while the tumor stroma was largely negative for the staining.	
Flow Cytometry: Flt-3/Flk-2/CD135 Antibody (7E8.2C8) [NBP2-42210] - HEK293 cells were stained (surface) with Flt-3/Flk-2/CD135 antibody (clone: 7E8.2C8; red) or isotype control (mouse IgG2 lambda; blue) and positive staining observed using PE conjugated mouse anti-IgG(H+L) secondary antibody. Live cells (PPI negative) were gated for analysis	

Procedures

Western Blot protocol for Flt-3/Flk-2/CD135 Antibody (NBP2-42210)
Flt-3/Flk-2/CD135 Antibody (7E8.2C8):
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-FLT3 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 Flt-3/Flk-2/CD135 Antibody (NBP2-42210)

Flt-3/Flk-2/CD135 Antibody (7E8.2C8):

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 NBP2-42210

NBP2-11587	Flt-3/Flk-2/CD135 Overexpression Lysate
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP2-42210AF488	Flt-3/Flk-2/CD135 Antibody (7E8.2C8) [Alexa Fluor® 488]

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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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