Product Datasheet

BST2 Antibody (4F6) - BSA Free
NBP2-29622

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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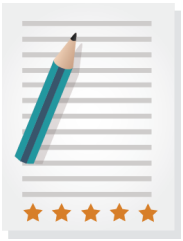


NBP2-29622

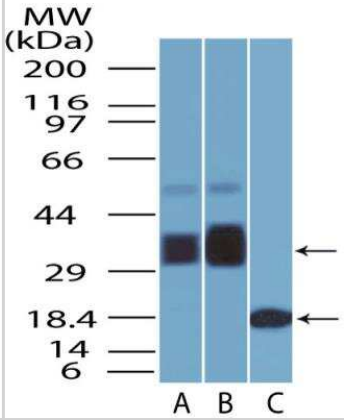
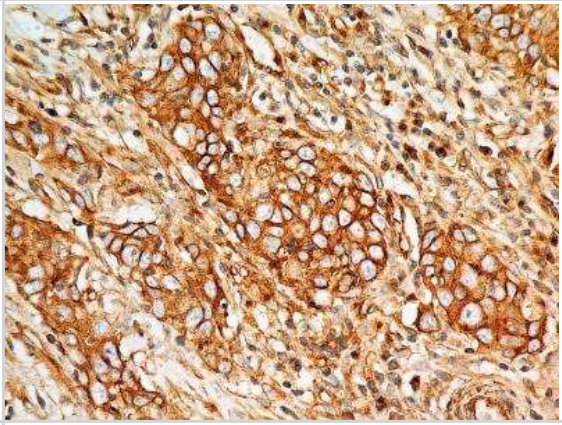
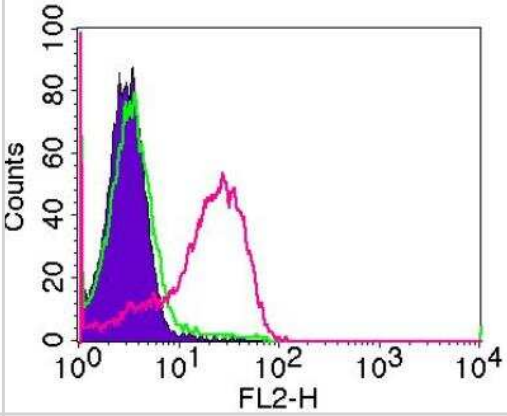
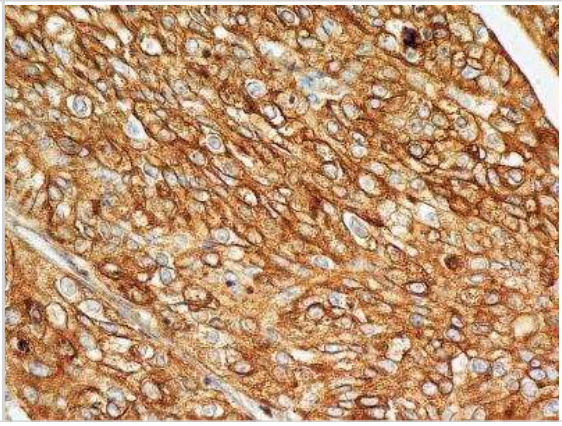
BST2 Antibody (4F6) - BSA Free

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	4F6
Preservative	0.05% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein A or G purified
Buffer	PBS

Product Description	
Host	Mouse
Gene ID	684
Gene Symbol	BST2
Species	Human
Immunogen	Full length recombinant human BST2 protein [NCBI NP_004326].

Product Application Details	
Applications	Western Blot, Flow Cytometry, Flow (Cell Surface), Immunohistochemistry, Immunohistochemistry-Paraffin, CyTOF-ready
Recommended Dilutions	Western Blot 1.0 ug/mL, Flow Cytometry 0.5 ug/mL, Immunohistochemistry 5 ug/mL, Immunohistochemistry-Paraffin 5 ug/mL, Flow (Cell Surface), CyTOF-ready
Application Notes	The unprocessed form of BST2 protein is 180 amino acids long (161 amino acids in the cleaved BST) and its cleaved form undergoes further modifications namely disulfide bond formation, GPI-anchor/GPI-like-anchor binding, glycosylation, isopeptide bond formation, lipidation and Ubl conjugation. The predicted molecular weight of unmodified BST2 protein is ~17-18 kDa, whereas the post-translationally modified protein has been documented to run at ~25-36 kDa (glycosylated), 50-55 kDa (ubiquitinated) and 65-70 kDa (dimers). BST2 protein localizes mainly to the cell membranes from where it shuttles to the trans-Golgi network, late endosome and the cytoplasm. In immuno-staining assays, BST2 may develop a membrane-cytoplasmic staining pattern. This antibody is CyTOF ready.

Images

Western Blot: BST2 Antibody (4F6) [NBP2-29622] - WB analysis of BST2 protein in (A) human heart lysate (B) human ovary lysate and on (C) partial recombinant BST2 protein with monoclonal BST2 antibody (clone 4F6) at a concentration of 1 ug/mL. In the tested lysates, two bands were observed which represents the glycosylated (lower intense bands) and the ubiquitinated (higher weak bands) forms of BST2 protein.	
Immunohistochemistry-Paraffin: BST2 Antibody (4F6) [NBP2-29622] - Analysis of BST2 protein in a section of human lung cancer (squamous cell carcinoma) using 5 ug/mL concentration of BST2 antibody (clone 4F6). The carcinoma cells depicted strong membrane-cytoplasmic BST2 positivity with more intense staining in the cellular membranes, and a relatively less intense staining pattern was observed in the tumor stroma.	
Flow (Cell Surface): BST2 Antibody (4F6) [NBP2-29622] - Flow analysis of Molt4 cells using the BST2 antibody [4F6]. The shaded purple region represents cells alone, green represents the isotype control and pink represents the BST2 antibody.	
Immunohistochemistry-Paraffin: BST2 Antibody (4F6) [NBP2-29622] - Analysis of BST2 protein in a section of human endometrial carcinoma using 5 ug/mL concentration of BST2 antibody (clone 4F6). The carcinoma cells depicted distinct membrane-cytoplasmic BST2 positivity with more intense staining in the cellular membranes.	

Procedures

Western Blot protocol for BST2 Antibody (NBP2-29622)
BST2 Antibody (4F6):
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-BST2 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 BST2 Antibody (NBP2-29622)

BST2 Antibody (4F6):

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 NBP2-29622

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)
NBP2-29622AF405	BST2 Antibody (4F6) [Alexa Fluor® 405]

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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