BOSTER BIOLOGICAL TECHNOLOGY
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Anti-NSF Antibody Picoband®

Catalog Number: A00585-1

About NSF

N-ethylmaleimide-sensitive factor, also known as NSF, is an enzyme which in humans is encoded by the NSF gene. NSF is a homohexameric AAA ATPase involved in membrane fusion. NSF is ubiquitously found in the cytoplasm of eukaryotic cells. It is a central component of the cellular machinery in the transfer of membrane vesicles from one membrane compartment to another. During this process, SNARE proteins on two joining membranes (usually a vesicle and a target membrane such as the plasma membrane) form a tight complex. This aids fusion of the vesicle with the target membrane. It has been proposed that the role of NSF is to undo these SNARE complexes once membrane fusion has occurred, using the hydrolysis of ATP as an energy source, allowing the dissociated SNAREs to be recycled for reuse in further rounds of membrane fusion. This proposal remains controversial, however. Recent work indicates that the ATPase function of NSF does not function in recycling of vesicles but rather functions in the act of fusing vesicles with the plasma membrane.

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Overview

Product Name	Anti-NSF Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-NSF Antibody Picoband® catalog # A00585-1. Tested in ELISA, Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	ELISA, Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4.
Storage Instructions	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	P46459

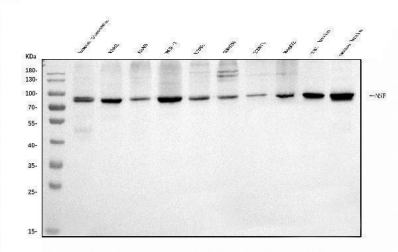
Technical Details

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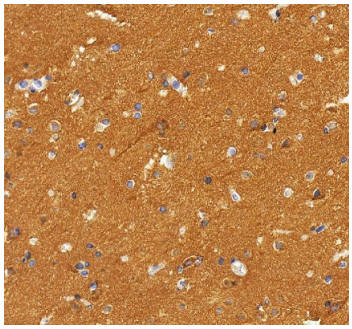
Immunogen	E.coli-derived human NSF recombinant protein (Position: R4-Q636).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.25ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 5ug/ml, Human Flow Cytometry (Fixed), 1-3ug/1x10^6 cells, Human ELISA, 0.1-0.5ug/ml, - Note: For flow cytometry (FCM), suitable for fixed cells only. Not suitable for FACS or surface flow cytometry using live cells.

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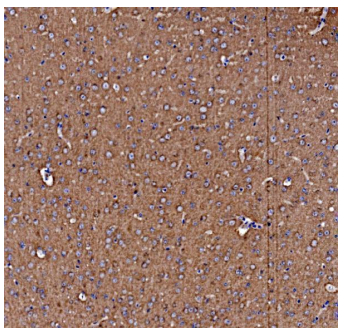
Anti-NSF Antibody Picoband® (A00585-1) Images



Western blot analysis of NSF using anti-NSF antibody (A00585-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human placenta tissue lysates, Lane 2: human K562 whole cell lysates, Lane 3: human A549 whole cell lysates, Lane 4: human MCF-7 whole cell lysates, Lane 5: human U20S whole cell lysates, Lane 6: human SW620 whole cell lysates, Lane 7: human 22RV1 whole cell lysates, Lane 8: human HepG2 whole cell lysates, Lane 9: rat brain tissue lysates, Lane 10: mouse brain tissue lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-NSF antigen affinity purified polyclonal antibody (Catalog # A00585-1) at 0.25 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for NSF at approximately 82 kDa. The expected band size for NSF is at 82 kDa.



IHC analysis of NSF using anti-NSF antibody (A00585-1). NSF was detected in a paraffin-embedded section of human brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-NSF Antibody (A00585-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

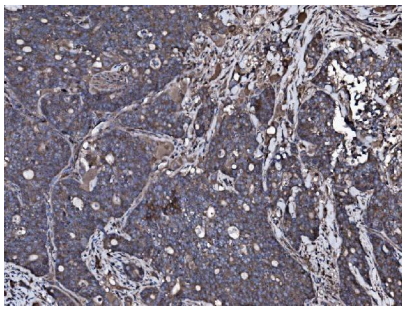


IHC analysis of NSF using anti-NSF antibody (A00585-1). NSF was detected in paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-NSF Antibody (A00585-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

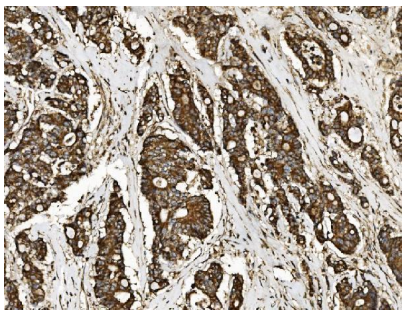
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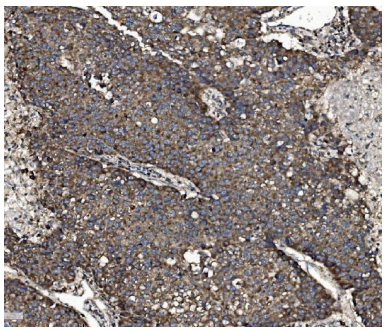
IHC analysis of NSF using anti-NSF antibody (A00585-1). NSF was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-NSF Antibody (A00585-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.



IHC analysis of NSF using anti-NSF antibody (A00585-1). NSF was detected in paraffin-embedded section of human gallbladder adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-NSF Antibody (A00585-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.



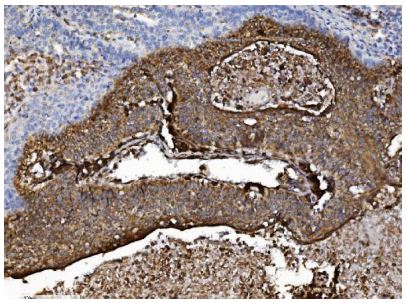
IHC analysis of NSF using anti-NSF antibody (A00585-1). NSF was detected in paraffin-embedded section of human colonic adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-NSF Antibody (A00585-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.



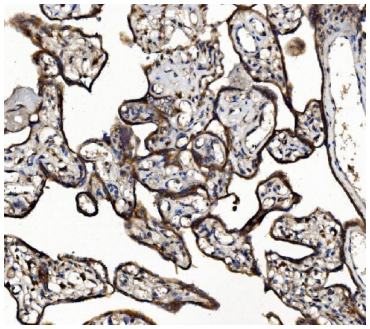
IHC analysis of NSF using anti-NSF antibody (A00585-1). NSF was detected in paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-NSF Antibody (A00585-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

IHC analysis of NSF using anti-NSF antibody (A00585-1). NSF was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-NSF Antibody (A00585-1) overnight at 4°C. Biotinylated goat

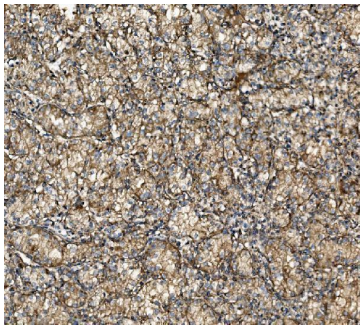
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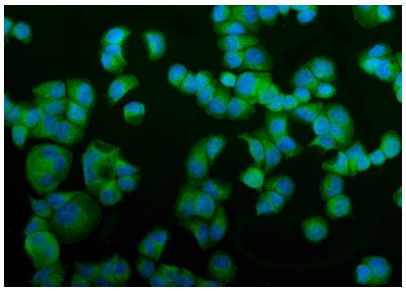
anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.



IHC analysis of NSF using anti-NSF antibody (A00585-1). NSF was detected in paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-NSF Antibody (A00585-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.



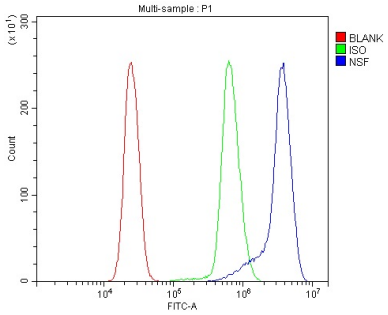
IHC analysis of NSF using anti-NSF antibody (A00585-1). NSF was detected in paraffin-embedded section of human renal clear cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-NSF Antibody (A00585-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.



IF analysis of NSF using anti-NSF antibody (A00585-1). NSF was detected in immunocytochemical section of T-47D cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5ug/mL rabbit anti-NSF Antibody (A00585-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

Flow Cytometry analysis of U937 cells using anti-NSF antibody (A00585-1). Overlay histogram showing U937 cells stained with A00585-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-NSF Antibody (A00585-1, 1ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit

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IgG (BA1127, 5-10ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x106) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

Anti-NSF Antibody

For Research Use Only. Not for use in diagnostic procedures.