Product Datasheet

beta-Actin Antibody (8H10D10) - BSA Free
NBP1-47423

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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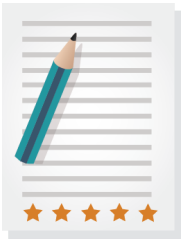


NBP1-47423

beta-Actin Antibody (8H10D10) - BSA Free

Product Information

Unit Size	0.1 ml
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	8H10D10
Preservative	0.02% Sodium Azide
Isotype	IgG2b
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	42 kDa

Product Description

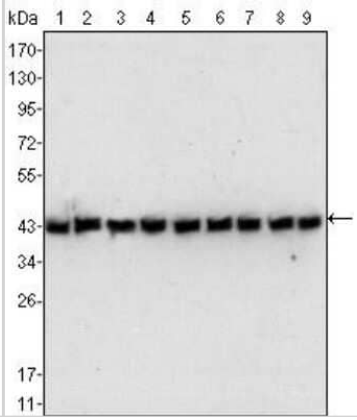
Host	Mouse
Gene ID	60
Gene Symbol	ACTB
Species	Human, Mouse, Rat, Chinese Hamster, Primate
Immunogen	This beta-Actin Antibody (8H10D10) was made to a synthetic peptide corresponding to amino-terminal residues of human Beta Actin, conjugated to KLH. [UniProt# P60709]

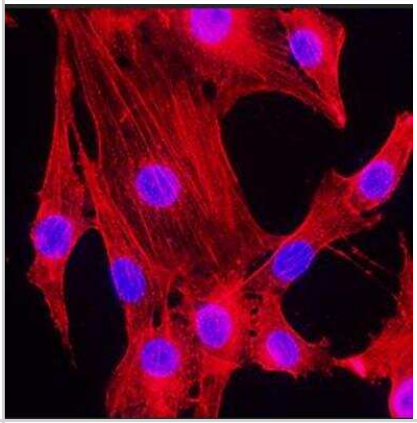
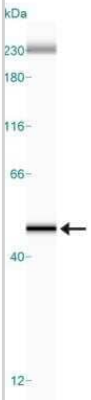
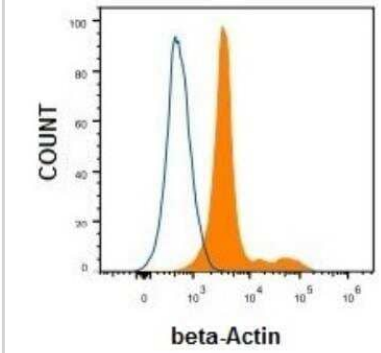
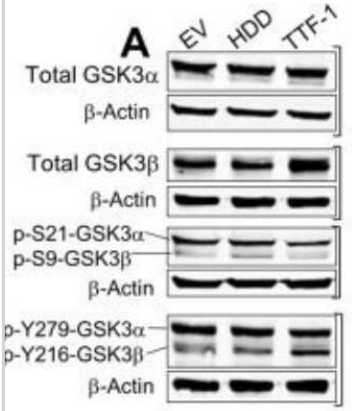
Product Application Details

Applications	Western Blot, Simple Western, ELISA, Flow Cytometry, Flow (Intracellular), Immunoblotting, Immunocytochemistry/ Immunofluorescence
Recommended Dilutions	Western Blot 1:1000-1:5000, Simple Western 12.5, Flow Cytometry 1:200-1:400, ELISA 1:10000, Immunocytochemistry/ Immunofluorescence 1:200-1:1000, Immunoblotting reported in scientific literature (PMID 27903531), Flow (Intracellular) 1:200 - 1:400
Application Notes	In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 1.0 mg/mL, separated by Size, antibody dilution of 12.5, apparent MW was 49 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

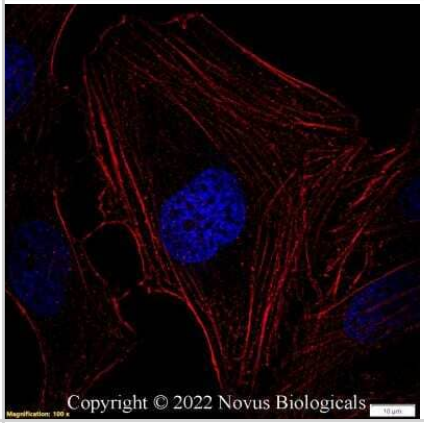
Images

Western Blot: beta-Actin Antibody (8H10D10) [NBP1-47423] - Analysis using anti-Beta Actin mAb against human, mouse, hamster, rat, and primate cell lystate(s): (1) NIH/3T3, (2) Jurkat, (3) HeLa, (4) CHO, (5) PC12, (6) HEK293, (7) COS, (8) A549, and (9) MCF-7. A specific band can be observed at a molecular weight of approximately 43 kDa in all lanes.

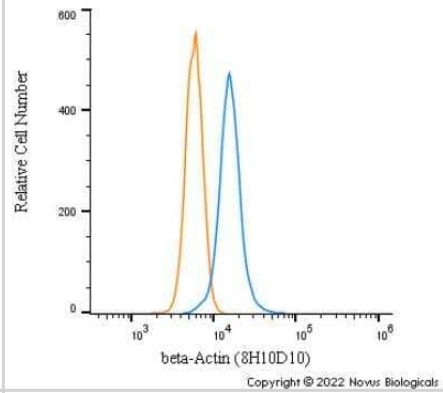


Immunocytochemistry/Immunofluorescence: beta-Actin Antibody (8H10D10) [NBP1-47423] - Beta actin was detected in NIH-3T3 cells fixed with methanol using mouse anti-mouse beta-Actin monoclonal antibody (NBP1-47423) at 1:5400 dilution. Cells were stained using NorthernLights 557 conjugated anti-mouse secondary antibody (NL007) and counterstained with DAPI.	
Simple Western: beta-Actin Antibody (8H10D10) [NBP1-47423] - Image shows a specific band for Beta Actin in 1.0 mg/mL of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Flow Cytometry: beta-Actin Antibody (8H10D10) [NBP1-47423] - Analysis of HeLa cells using mouse Monoclonal beta-Actin antibody (Orange) and Isotype control Antibody (Blue).	
Western Blot: beta-Actin Antibody (8H10D10) [NBP1-47423] - TTF-1 alters the phosphorylation of GSK3alpha/beta. (A) Panels of immunoblot images examining total GSK3alpha/beta and phosphorylation at specific Ser or Tyr residues in A549 transfectant cells. Beta-Actin is included as a loading control. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41598-019-44549-w), licensed under a CC-BY license.	

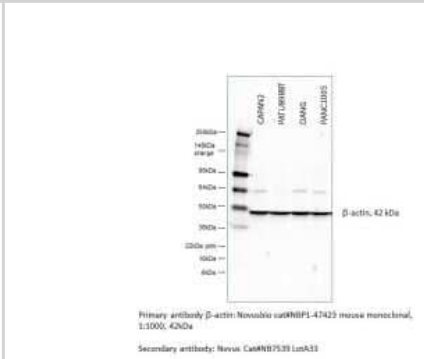
Immunocytochemistry/Immunofluorescence: beta-Actin Antibody (8H10D10) [NBP1-47423] - HeLa cells were fixed and permeabilized for 10 minutes with -20C MeOH. The cells were incubated with beta-Actin Antibody [8H10D10] conjugated to Biotin (NBP1-47423B) at 5ug/ml overnight at 4C and detected with Streptavidin conjugated to DyLight 550. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



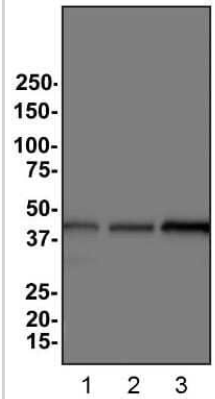
Flow Cytometry: beta-Actin Antibody (8H10D10) - BSA Free [NBP1-47423] - An intracellular stain was performed on NIH3T3 cells with beta-Actin Antibody (8H10D10) NBP1-47423 (blue) and a matched isotype control MAB004 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (84540, Thermo Fisher).

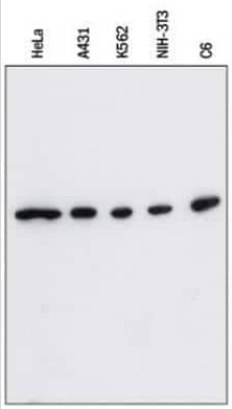
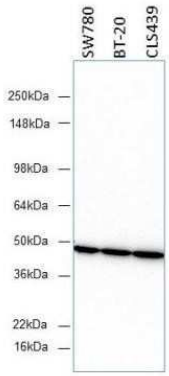
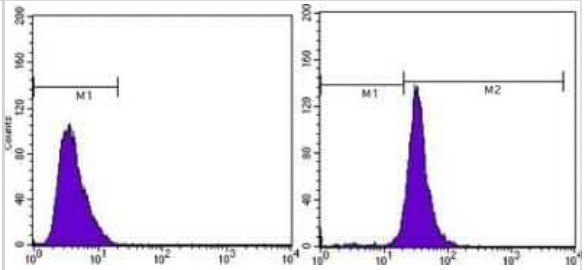
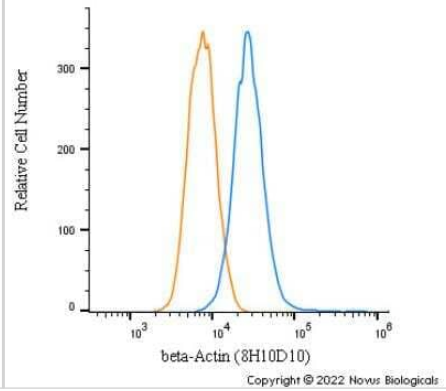


Western Blot: beta-Actin Antibody (8H10D10) [NBP1-47423] - Western blot of pancreatic cell lines (CAPAN2, PATU8988T, DANG, and PANC1005). Primary antibody: beta-Actin (8H10D10), NBP1-47423, 1:1000 dilution. Secondary antibody: HRP conjugated Mouse IgG (H+L), NB7539. A specific band can be observed at a molecular weight of approximately 42 kDa. Image from verified customer review.

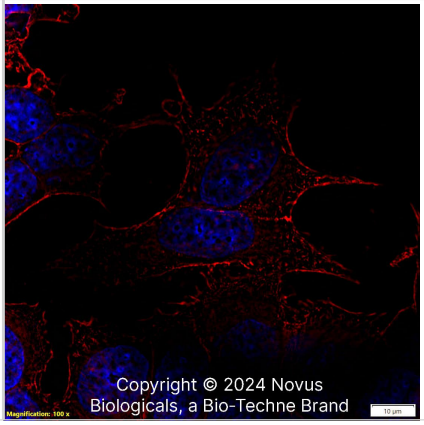


Western Blot: beta-Actin Antibody (8H10D10) [NBP1-47423] - Analysis of Beta Actin expression in 1) HeLa 2) HepG2 and 3) Cos7 whole cell lysates.



Western Blot: beta-Actin Antibody (8H10D10) [NBP1-47423] - Lysates of HeLa human cervical epithelial carcinoma cell line, A431 human epithelial carcinoma cell line, K562 human chronic myelogenous leukemia cell line, NIH-3T3 mouse embryonic fibroblast cell line, and C6 rat glioma cell line were probed with 1:2500 mouse anti-beta-Actin monoclonal (NBP1-47423) followed by 1:2000 dilution of donkey anti-mouse IgG-HRP secondary antibody (HAF018).	
Western Blot: beta-Actin Antibody (8H10D10) [NBP1-47423] - Three different human cancer cell lines (SW780, BT-20, and CLS439) were probed with the antibody. Image from verified customer review.	
Flow Cytometry: beta-Actin Antibody (8H10D10) [NBP1-47423] - Analysis of MCF-7 cells using anti-Beta Actin mAb (right) and negative control (left).	
Flow Cytometry: beta-Actin Antibody (8H10D10) - BSA Free [NBP1-47423] - An intracellular stain was performed on U-251 MG cells with beta-Actin Antibody (8H10D10) NBP1-47423 (blue) and a matched isotype control MAB004 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (84540, Thermo Fisher).	

Beta-Actin (8H10D10) was detected in immersion fixed MCF7 human breast cancer cell line using Mouse anti-beta-Actin (8H10D10) Protein G Purified Monoclonal Antibody conjugated to Biotin (Catalog # NBP1-47423B) at 5 µg/mL overnight at 4C. Cells were stained using Streptavidin conjugated to DyLight 550 (red) and counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



Publications

Li J, Jeong SY, Xiong B et Al. Repurposing pyridoxamine for therapeutic intervention of intravascular cell-cell interactions in mouse models of sickle cell disease Haematologica 2019-10-31 [PMID: 33054081]

S Nemati, H Mohammad R, A Meyfour, H Pazoki, H Asadzadeh, S Shahrokh, H Mirjalali Evaluation of the mTORC activity in the presence of Toxoplasma gondii and azathioprine in human monocyte cell line BMC microbiology, 2023-03-21;23(1):77. 2023-03-21 [PMID: 36941573]

James Elste, Nicole Cast, Shalini Udawatte, Kabita Adhikari, Shannon Harger Payen, Subhash C Verma, Deepak Shukla, Michelle Swanson-Mungerson, Vaibhav Tiwari Co-Expression of Niemann-Pick Type C1-Like1 (NPC1L1) with ACE2 Receptor Synergistically Enhances SARS-CoV-2 Entry and Fusion. Biomedicines 2024-04-08 [PMID: 38672177]

Enes Akkaya, Şevket Evran, Fatih Çalış, Serdar Çevik, Salim Katar, Ersin Karataş, Abdurrahim Koçyiğit, Mustafa Yasin Sağlam, Mustafa Aziz Hatiboğlu, Hakan Hanımoğlu, Mehmet Yaşar Kaynar Thymoquinone ameliorates delayed cerebral injury and cerebral vasospasm secondary to experimental subarachnoid haemorrhage. Neurologia i neurochirurgia polska 2021-01-07 [PMID: 33252137]

Azimirad M, Noori M, Amirkamali S et al. Clostridioides difficile PCR ribotypes 001 and 084 can trigger autophagy process in human intestinal Caco-2 cells Microbial pathogenesis 2023-11-16 [PMID: 37979713] (WB)

Weldemariam MM, Woo J, Zhang Q. Pancreatic INS-1 ?-Cell Response to Thapsigargin and Rotenone: A Comparative Proteomics Analysis Uncovers Key Pathways of ?-Cell Dysfunction Chemical Research in Toxicology 2022-06-20 [PMID: 35544339] (Western Blot)

Hassan MH, Abuhamdah S, Elsadek BEM et al. Expression Patterns of Macrophage Migration Inhibitory Factor and Its Gene Variants (MIF-173 G?C) in Verruca Vulgaris Clinical, cosmetic and investigational dermatology 2022-06-10 [PMID: 35712358] (WB, Human)

Details:
Dilution: 1:5000

Martin S, Mu D CRISPR-Induced Loss of Connexin 43 Expression Sensitizes KRAS Mutant Cells to Cisplatin microPublication biology 2022-11-13 [PMID: 36447529] (WB, Human)

Amirkamali S, Azimirad M, Nasiri G et al. Surface layer protein A from hypervirulent Clostridioides difficile ribotype 001 can induce autophagy process in human intestinal epithelial cells Microbial pathogenesis 2022-07-15 [PMID: 35850375]

Badr El-Din NK, Othman AI, Amer ME, Ghoneum M Thymax, a gross thymic extract, exerts cell cycle arrest and apoptosis in Ehrlich ascites carcinoma in vivo Heliyon 2022-03-01 [PMID: 35299600] (WB, Mouse)

Abe, R J, Savage, H Et al. p90RSK-MAGI1 Module Controls Endothelial Permeability by Post-translational Modifications of MAGI1 and Hippo Pathway. Front Cardiovasc Med 2020-12-12 [PMID: 33304925] (Simple Western, Mouse)

Guan J, Lu C, Jin Q et al. MLH1 Deficiency-Triggered DNA Hyperexcision by Exonuclease 1 Activates the cGAS-STING Pathway Cancer cell 2020-12-03 [PMID: 33338427]

More publications at http://www.novusbio.com/NBP1-47423

Procedures

Western Blot Protocol for beta-Actin Antibody (NBP1-47423)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST 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.

Flow (Intracellular) Protocol for beta-Actin Antibody (NBP1-47423)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.

a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.

4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeablization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.

a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.

b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).

3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

Immunohistochemistry-Paraffin Protocol for beta-Actin Antibody (NBP1-47423)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer all the time).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

Immunocytochemistry/Immunofluorescence Protocol for beta-Actin Antibody (NBP1-47423)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

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Products Related to NBP1-47423

NB800-PC1	HeLa Whole Cell Lysate
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP2-27231	Mouse IgG2b Isotype Control (MPC-11)

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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