Product Datasheet

beta-Catenin Antibody (12F7) - BSA Free
NBP1-54467

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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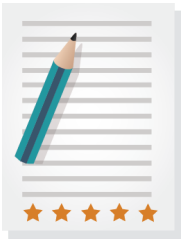
Updated 4/13/2025 v.20.1



NBP1-54467

beta-Catenin Antibody (12F7) - BSA Free

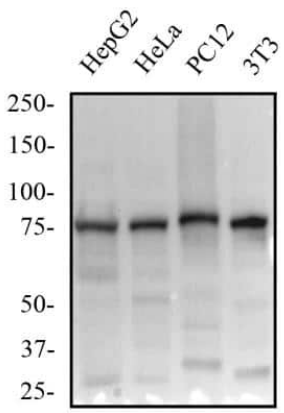
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	12F7
Preservative	0.02% Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	92 kDa

Product Description	
Host	Mouse
Gene ID	1499
Gene Symbol	CTNNB1
Species	Human, Mouse, Rat, Duck, Chicken, Primate
Reactivity Notes	Use in Duck reported in scientific literature (PMID:32692763).
Marker	Epithelial Cell Marker, Adherens Junction Marker
Immunogen	Recombinant chicken beta Catenin fused to maltose binding protein. [UniProt# O42486]

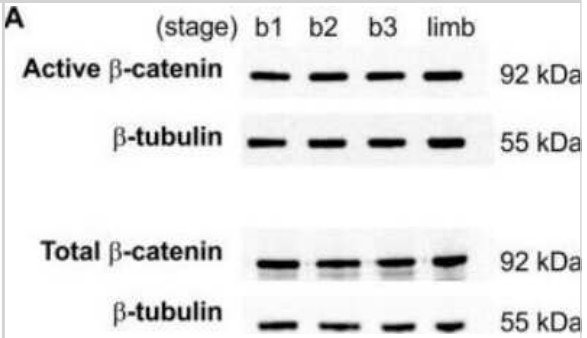
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:100, Flow Cytometry, Immunohistochemistry 1:100-1:200, Immunocytochemistry/ Immunofluorescence 1:50-1:100, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:100-1:200, Flow (Intracellular)
Application Notes	This beta Catenin (12F7) antibody is useful for IHC-P sections, ICC/IF, IP and WB, where a band can be seen at approx. 92 kDa. 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 HepG2 lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:100, apparent MW was 90 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

Images

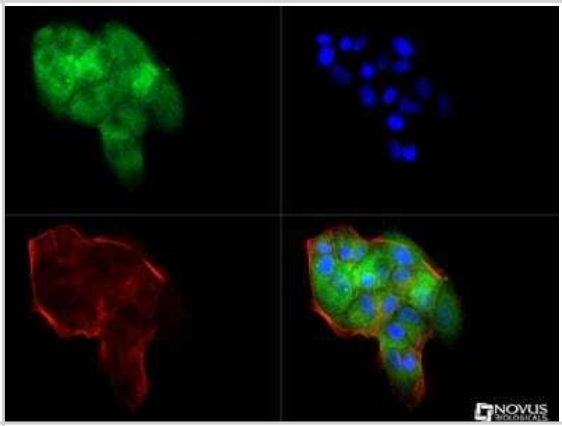
Western Blot: beta-Catenin Antibody (12F7) [NBP1-54467] - Total protein from human HepG2 and HeLa cells, rat PC12 cells and mouse 3T3 cells was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 1.0 ug/ml anti-Beta catenin in 1% non-fat milk in TBST and detected with an anti-mouse HRP secondary antibody using chemiluminescence.



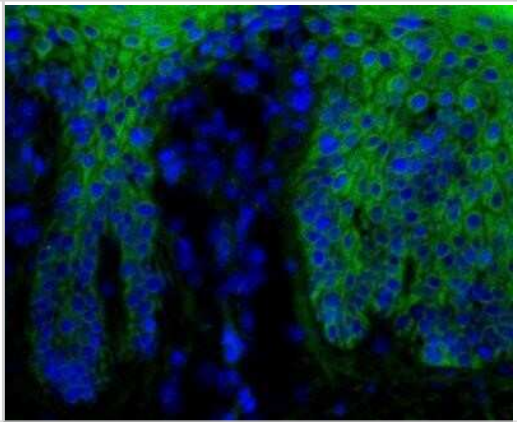
Western Blot: beta-Catenin Antibody (12F7) [NBP1-54467] - Activity of Wnt/beta-catenin pathway in the embryonic chick lung. Western blot analysis of active and total beta-catenin in stage b1, b2 and b3 lungs, and stage 24 limb (as positive control). Control loading was performed using beta-tubulin (55 kDa). Total and active beta-catenin correspond to 92 kDa. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0112388), licensed under a CC-BY license.



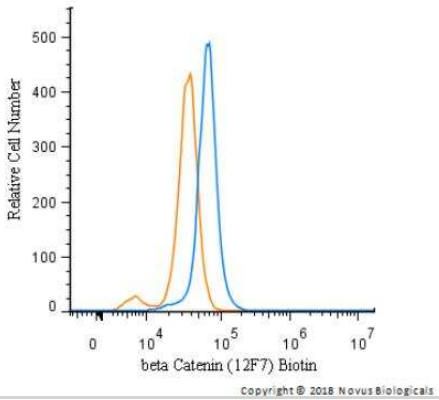
Immunocytochemistry/Immunofluorescence: beta-Catenin Antibody (12F7) [NBP1-54467] - The beta- Catenin antibody was tested in MCF-7 cells against Dylight 488 (Green). Actin and nuclei were counterstained against Phalloidin 550 (Red) and DAPI (Blue).



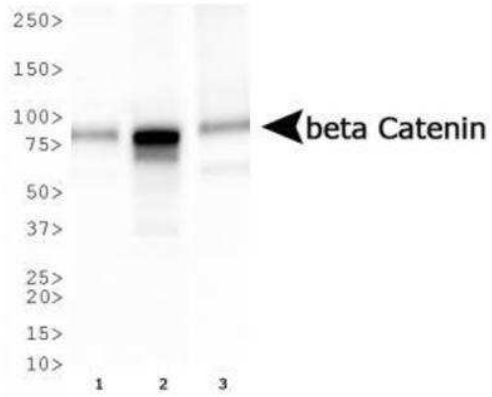
Immunohistochemistry: beta-Catenin Antibody (12F7) [NBP1-54467] - Beta-catening (green) was detected in human skin (psoriasis) using beta-catenin-FITC antibody (1:40) in PBS for 1 hour. Nuclei were stained with Dapi (blue). Image from verified customer review. Image using the FITC format of this antibody.



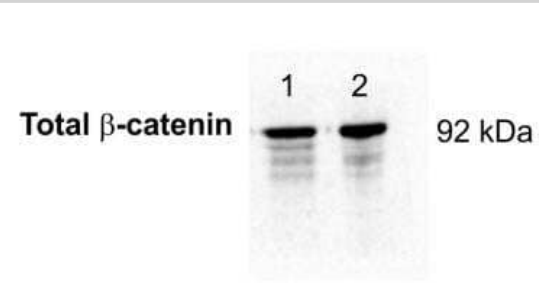
Flow Cytometry: beta-Catenin Antibody (12F7) [NBP1-54467] - An intracellular stain was performed on SK-MEL-28 cells with beta-Catenin Antibody [12F7] NBP1-54467B (blue) and a matched isotype control (orange). Both antibodies were conjugated to Biotin. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Streptavidin - R-Phycoerythrin Protein (2012-1000, Novus Biologicals).



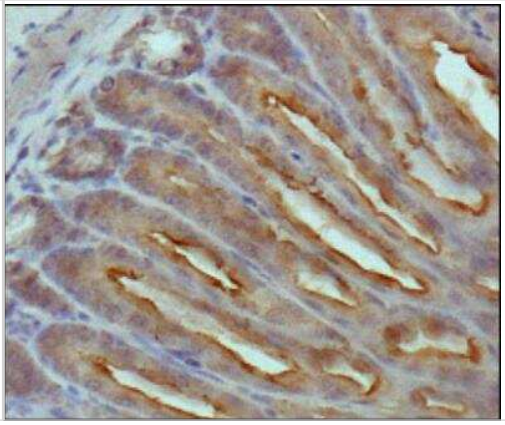
Western Blot: beta-Catenin Antibody (12F7) [NBP1-54467] - Analysis of beta Catenin expression in 1) HepG2, 2) MCF7, and 3) Cos7 whole cell lysates using NBP1-54467.



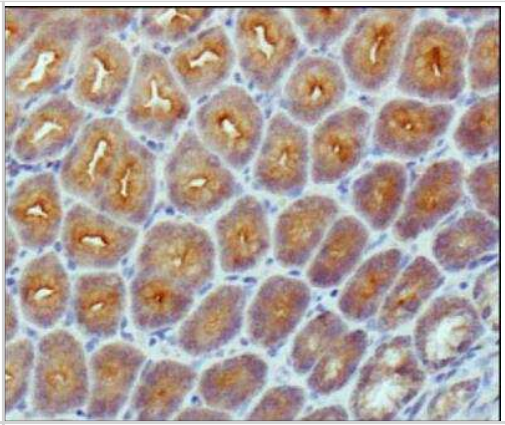
Western Blot: beta-Catenin Antibody (12F7) [NBP1-54467] - Analysis of beta- Catenin in embryonic lung (lane 1) and embryonic limb (lane 2) lysates using anti-beta- Catenin antibody. Each lane was loaded with 5ug of protein sample. Image from verified customer review.



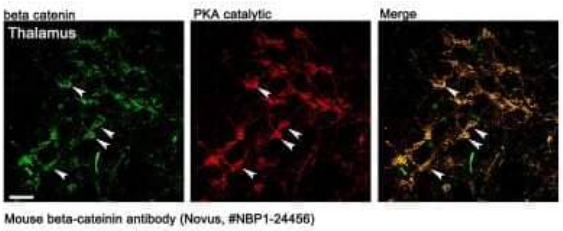
Immunohistochemistry-Paraffin: beta-Catenin Antibody (12F7) [NBP1-54467] - Analysis of beta Catenin in mouse intestine using DAB with hematoxylin counterstain.



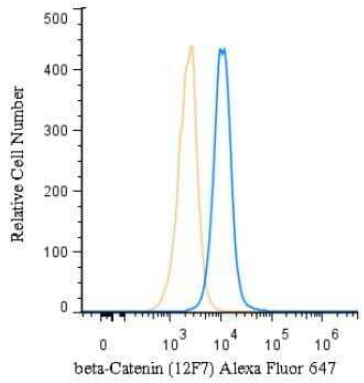
Immunohistochemistry-Paraffin: beta-Catenin Antibody (12F7) [NBP1-54467] - Analysis of beta- Catenin in mouse intestine using DAB with hematoxylin counterstain.



Immunohistochemistry: beta-Catenin Antibody (12F7) [NBP1-54467] - IF beta-Catenin staining of mouse brain tissue. This image submitted by a verified customer review.

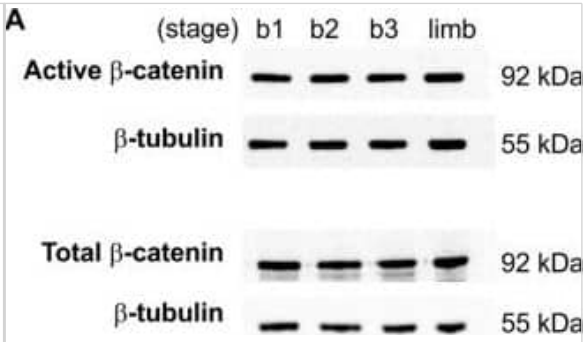


Flow (Intracellular): beta-Catenin Antibody (12F7) [NBP1-54467] - An intracellular stain was performed on HeLa cells with beta-Catenin Antibody (12F7) NBP1-54467AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



Simple Western: beta-Catenin Antibody (12F7) [NBP1-54467] - Simple Western lane view shows a specific band for Beta- Catenin in 0.5 mg/ml of HepG2 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

Activity of Wnt/β-catenin pathway in the embryonic chick lung.(A) Western blot analysis of active and total β-catenin in stage b1, b2 and b3 lungs, and stage 24 limb (as positive control). Control loading was performed using β-tubulin (55 kDa). Total and active β-catenin correspond to 92 kDa. (B) Semi-quantitative analysis for active and total β-catenin. Results are presented as arbitrary units normalized for β-tubulin. p<0.05: * vs limb.



Publications

Shin-Ichiro Hino, Kiyoka Inenaga, Takuto Miyazaki, Chika Tanaka-Mizota Suppression of HCT116 Human Colon Cancer Cell Motility by Polymethoxyflavones is Associated with Inhibition of Wnt/β-Catenin Signaling. Nutrition and cancer 2022-09-08 [PMID: 35658755]

Aamir K, Sethi G, Afrin MR et al. Arjunolic acid modulate pancreatic dysfunction by ameliorating pattern recognition receptor and canonical Wnt pathway activation in type 2 diabetic rats Life sciences 2023-08-15 [PMID: 37307966] (Simple Western, IHC, Rat)

Urasaki Y, Le TT Functional Complementation of Anti-Adipogenic Phytonutrients for Obesity Prevention and Management Nutrients 2022-10-16 [PMID: 36297009] (WB, Human)

Bhatia R, Thompson CM, Clement EJ et al. Malondialdehyde-Acetaldehyde Extracellular Matrix Protein Adducts Attenuate Unfolded Protein Response During Alcohol and Smoking-Induced Pancreatitis Gastroenterology 2022-07-03 [PMID: 35788346] (ICC/IF)

Details:
Fig, 6E.

Cameron S Anti-Cancer and Stress Response Pathway Effects of Nanosilver and Sodium Ascorbate Carleton University 2022-07-11 (WB, Human)

Clinch M The Role of Hypoxia on PORCN and WLS Expression in Human Embryonic Kidney (HEK293T) and Human Colon Cancer (HCT-116T) Cells Carleton University 2022-04-05 (WB, Human)

Sheng J Cellular Effects Nanosilver on Cancer and Non-cancer Cells: Potential Environmental and Human Health Impacts Thesis

Sidor J, Gillette M, Dezi L et al. Role of Presenilin-1 in Aggressive Human Melanoma International Journal of Molecular Sciences 2022-04-28 [PMID: 35563300] (WB, Human)

Urasaki, Y, Beaumont, C Et al. Potency Assessment of CBD Oils by Their Effects on Cell Signaling Pathways. Nutrients 2020-01-30 [PMID: 32019055] (WB, Human)

Gul HF, Ilhan N, Ilhan N et al. The Combined Effect of Pomegranate Extract and Tangeretin on the DMBA-induced Breast Cancer Model The Journal of nutritional biochemistry 2020-12-13 [PMID: 33326843] (WB, Rat)

GonCalves AN, Correia-Pinto J, Nogueira-Silva C ROBO2 signaling in lung development regulates SOX2/SOX9 balance, branching morphogenesis and is dysregulated in nitrofen-induced congenital diaphragmatic hernia Respir Res 2020-11-18 [PMID: 33208157] (WB)

Liu Z, Selby CP, Yang Y et al. Circadian regulation of c-MYC in mice Proc. Natl. Acad. Sci. U.S.A. 2020-08-19 [PMID: 32817420] (WB, Mouse)

More publications at http://www.novusbio.com/NBP1-54467

Procedures

Western Blot protocol for beta-Catenin Antibody (NBP1-54467)

Western Blot Protocol
1. Perform SDS-PAGE on samples to be analyzed, loading 40 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 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%.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Immunohistochemistry-Paraffin protocol for beta-Catenin Antibody (NBP1-54467)

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.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution 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 biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

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Immunocytochemistry/ Immunofluorescence Protocol for beta-Catenin Antibody (NBP1-54467)

Immunocytochemistry Protocol

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

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Flow (Intracellular) Protocol for beta-Catenin Antibody (NBP1-54467)

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 1 mL 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:

Optional: Perform cell surface staining as described in the previous section.

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 5 minutes at 400 RCF.
5. Discard supernatant and re-suspend in 1 mL of staining buffer + 0.1% permeabilizer.
6. Stain each sample at 1 uL/ 1 x 106 cells of primary antibody or 1-3 uL/ 1 x 106 cells for directly conjugated antibodies. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
7. Following the primary/conjugate incubation, add 2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 5 minutes at 400 RCF.
8. Remove supernatant and re-suspend each sample in 2 mL staining buffer + 0.1% permeabilizer, repeat wash for 5 minutes at 400 RCF.
9. If using a directly conjugated antibody, after the second wash, re-suspend cell pellet to a final volume of 500 uL per sample and proceed with flow analysis.

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Products Related to NBP1-54467

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)
NBP2-61628PEP	beta-Catenin Recombinant Protein Antigen

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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