Product Datasheet

AMPK alpha 1 Antibody (2B7) - BSA Free
NBP2-22127

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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Publications: 12

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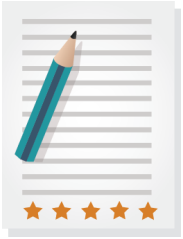
Updated 4/13/2025 v.20.1



NBP2-22127

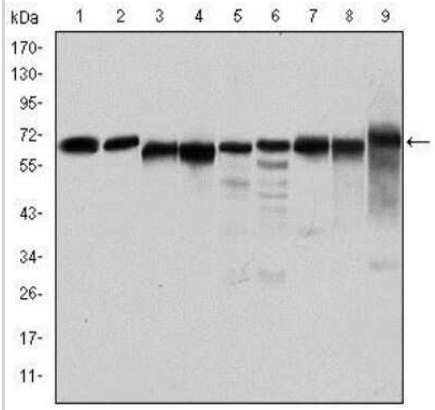
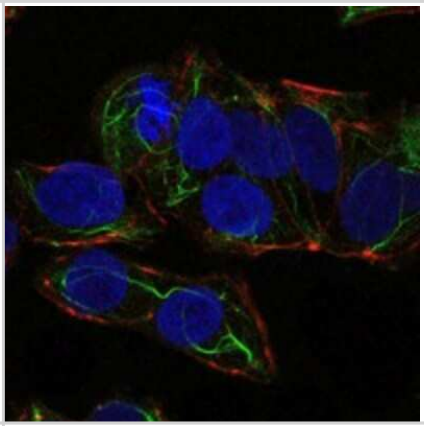
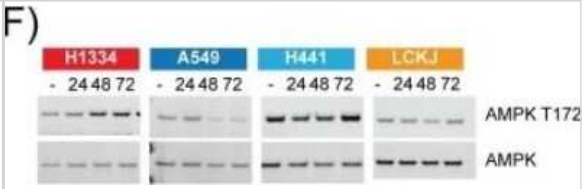
AMPK alpha 1 Antibody (2B7) - BSA Free

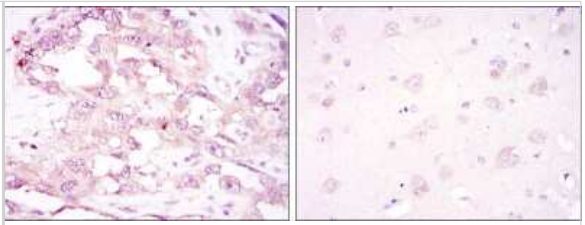
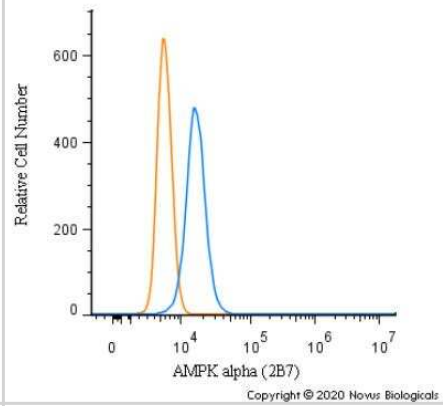
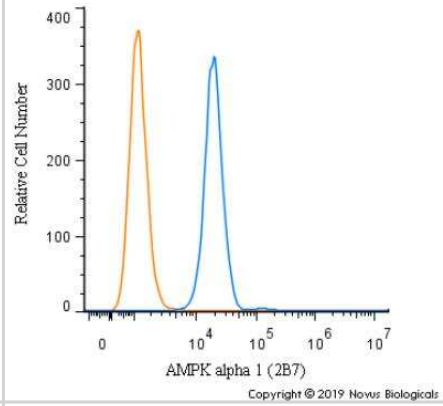
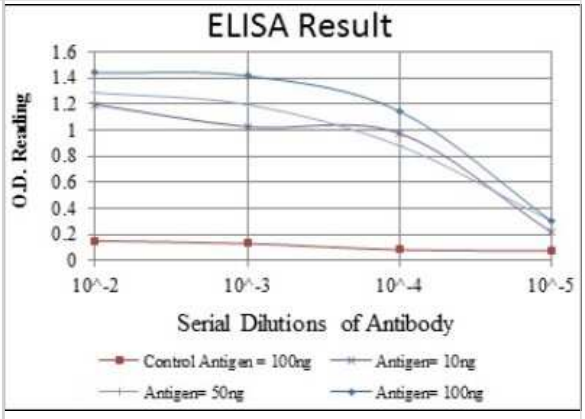
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	2B7
Preservative	0.02% Sodium Azide
Isotype	IgG1
Purity	Protein A or G purified
Buffer	PBS
Target Molecular Weight	64 kDa

Product Description	
Host	Mouse
Gene ID	5562
Gene Symbol	PRKAA1
Species	Human, Mouse, Rat, Primate
Immunogen	Purified recombinant fragment of human AMPK alpha 1 expressed in E. coli. [UniProt# Q13131]

Product Application Details	
Applications	Western Blot, Simple Western, ELISA, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:500, Simple Western 1:50, Flow Cytometry 1:200-1:400, ELISA 1:10000, Immunohistochemistry 1:200 - 1:1000, Immunocytochemistry/ Immunofluorescence 1:200-1:1000, Immunohistochemistry-Paraffin 1:200-1:1000
Application Notes	This AMPK alpha 1 (2B7) antibody is useful for Western blot, Immunohistochemistry on paraffin-embedded sections, Immunocytochemistry/Immunofluorescence, Flow Cytometry and ELISA. 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 1:50, apparent MW was 63 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

Images

Simple Western: AMPK alpha 1 Antibody (2B7) [NBP2-22127] - Simple Western lane view shows a specific band for AMPK Alpha 1 in 0.5 mg/ml of HepG2 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Western Blot: AMPK alpha 1 Antibody (2B7) [NBP2-22127] - Western blot analysis using AMPK alpha 1 mouse mAb against Jurkat (1), Hela (2), HepG2 (3), MCF-7 (4), Cos7 (5), NIH/3T3 (6), K562 (7), HEK293 (8), and PC-12 (9) cell lysate.	
Immunocytochemistry/Immunofluorescence: AMPK alpha 1 Antibody (2B7) [NBP2-22127] - Immunofluorescence analysis of NTERA-2 cells using AMPK alpha 1 mouse mAb (green). Blue: DRAQ5 fluorescent DNA dye. Red: Actin filaments have been labeled with Alexa Fluor-555 phalloidin.	
Western Blot: AMPK alpha 1 Antibody (2B7) [NBP2-22127] - Ability to combat ROS varies across cell lines. Western blot analysis showing levels of AMPK and activated AMPK (T172) at 24, 48 and 72 hours following exposure to 0.4 uM GNE-617. Full-length western blots are shown in S2D Fig. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0164166), licensed under a CC-BY license.	

Immunohistochemistry-Paraffin: AMPK alpha 1 Antibody (2B7) [NBP2-22127] - Immunohistochemical analysis of paraffin-embedded ovarian cancer (left) and brain tissues (right) using AMPK alpha 1 mouse mAb with DAB staining.	
Flow Cytometry: AMPK alpha 1 Antibody (2B7) [NBP2-22127] - An intracellular stain was performed on NIH3T3 cells with AMPK alpha 1 Antibody (2B7) NBP2-22127 (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 1.0 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher).	
Flow Cytometry: AMPK alpha 1 Antibody (2B7) [NBP2-22127] - An intracellular stain was performed on RH-30 cells with AMPK alpha 1 Antibody (2B7) NBP2-22127PE (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin.	
ELISA: AMPK alpha 1 Antibody (2B7) [NBP2-22127] - Red: Control Antigen (100ng); Purple: Antigen (10ng); Green: Antigen (50ng); Blue: Antigen (100ng).	

Publications

Nguyen K, Rivera A, Alzoubi M et al. Evaluation of liver kinase B1 downstream signaling expression in various breast cancers and relapse free survival after systemic chemotherapy treatment Oncotarget 2021-05-25 [PMID: 34084284] (Western Blot)

Jassim, A H, Cavanaugh, M Et al. Transcorneal Electrical Stimulation Reduces Neurodegenerative Process in a Mouse Model of Glaucoma. Ann Biomed Eng 2021-02-01 [PMID: 32974756] (ICC/IF, Human)

Mehanna ET, Khalaf SS, Mesbah NM Et al. Anti-oxidant, anti-apoptotic, and mitochondrial regulatory effects of selenium nanoparticles against vancomycin induced nephrotoxicity in experimental rats Life sciences 2021-10-26 [PMID: 34715137] (WB, Rat)

Chen Y, Duan Y et al. Inhibition of ERK1/2 and activation of LXR synergistically reduce atherosclerotic lesions in ApoE-deficient mice. Arterioscler Thromb Vasc Biol 2015-01-04 [PMID: 25810299] (WB, Human)

Harun-Or-Rashid M, Pappenhagen N, Zubricky R et al. MCT2 overexpression rescues metabolic vulnerability and protects retinal ganglion cells in two models of glaucoma Neurobiol. Dis. 2020-05-15 [PMID: 32422282] (WB, Mouse)

Vila IK, Park MK, Setijono SR et al A muscle-specific UBE2O/AMPK alpha 2 axis promotes insulin resistance and metabolic syndrome in obesity JCI Insight 2019-07-11 [PMID: 31292296] (WB, Mouse)

Korponay TC, Balnis J, Vincent CE et al. High CO2 downregulates skeletal muscle protein anabolism via AMPK alpha 2-mediated depressed ribosomal biogenesis Am. J. Respir. Cell Mol. Biol. 2019-07-02 [PMID: 31264907]

Harun-Or-Rashid M, Inman DM. Reduced AMPK activation and increased HCAR activation drive anti-inflammatory response and neuroprotection in glaucoma. J Neuroinflammation. 2018-11-13 [PMID: 30424795] (WB, Mouse)

Chauhan AS, Liu X, Jing J et al. STIM2 interacts with AMPk and regulates calcium-induced AMPk activation. FASEB J. 2018-10-18 [PMID: 30335546] (ICC/IF, Human)

Dai S, Dulcey AE, Hu X et al. Methyl-B-cyclodextrin restores impaired autophagy flux in Niemann-Pick C1-deficient cells through activation of AMPK. Autophagy. 2017-06-14 [PMID: 28613987] (WB, Human)

Vila IK, Yao Y, Kim G et al. A UBE2O-AMPKalpha2 Axis that Promotes Tumor Initiation and Progression Offers Opportunities for Therapy. Cancer Cell. 2017-02-13 [PMID: 28162974] (Mouse)

Xiao Y, Kwong M, Daemen A et al. Metabolic Response to NAD Depletion across Cell Lines Is Highly Variable. PLoS ONE 2016-10-06 [PMID: 27711204]

Procedures

Western Blot Protocol for AMPK alpha 1 Antibody (NBP2-22127)
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 instructi

Immunohistochemistry-Paraffin Protocol for AMPK alpha 1 Antibody (NBP2-22127)
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 AMPK alpha 1 Antibody (NBP2-22127)
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.

Flow (Intracellular) Protocol for AMPK alpha 1 Antibody (NBP2-22127)

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.

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Products Related to NBP2-22127

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)
NBP2-51992-0.1mg	Recombinant Human AMPK alpha 1 His Protein

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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