Datasheet for ABIN967930

anti-AP2B1 antibody (AA 75-245)

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Overview

Quantity:	50 µg
Target:	AP2B1
Binding Specificity:	AA 75-245
Reactivity:	Human, Mouse, Rat, Dog, Chicken, Frog
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This AP2B1 antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (IHC), Immunoprecipitation (IP), BioImaging (BI)

Product Details

Immunogen:	Human Adaptin beta aa. 75-245
Clone:	74-Adaptin beta
Isotype:	IgG1
Cross-Reactivity:	Mouse (Murine), Rat (Rattus), Dog (Canine), Chicken, Frog
Characteristics:	1. Since applications vary, each investigator should titrate the reagent to obtain optimal results. 2. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance. 3. Source of all serum proteins is from USDA inspected abattoirs located in the United States. 4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive

Product Details

	deposits in plumbing. 5. Please refer to us for technical protocols.
Purification:	The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Target Details

Target:	AP2B1
Alternative Name:	Adaptin beta (AP2B1 Products)
Background:	Sorting of integral membrane proteins at various stages of the endocytic and secretory pathways is mediated by vesicular trafficking between a variety of organelles. Two sorting signals are tyrosine-based and dileucine-based signals that interact with heterotetrameric adaptor protein complexes (AP-1, AP-2, AP-3, and AP-4), which are associated with the vesicle coats. These coatomers contain two large Adaptin proteins (gamma, alpha, delta, or epsilon and beta1, beta2, beta3, or beta4, respectively) that are noncovalently linked to one medium chain (µ1, µ2, µ3, or µ4) and one small chain (sigma1, sigma2, sigma3, or sigma4). The AP-1 and AP-3 complexes are involved in protein sorting from the TGN and endosomes, while AP-2 adaptor complexes are involved in clathrin-mediated endocytosis. beta Adaptin subunits (beta1, beta2, beta3, beta4) lack sequence homology to adaptins alpha, gamma, delta, and epsilon, but all of these subunits share a similar domain structure. Adaptin beta1 (also known as Adaptin beta') and beta2 (also known as Adaptin beta) have 83% amino acid identity and are found in the AP1 and AP2 complexes, respectively.
Molecular Weight:	106 kDa
Pathways:	EGFR Signaling Pathway, Neurotrophin Signaling Pathway, EGFR Downregulation

Application Details

Application Notes:	Bioimaging 1. Seed the cells in appropriate culture medium at ~10,000 cells per well in an 96-well Imaging Plate and culture overnight. 2. Remove the culture medium from the wells, and fix the cells by adding 100 myl of Fixation Buffer to each well. Incubate for 10 minutes at room temperature (RT). 3. Remove the fixative from the wells, and permeabilize the cells using either 90% methanol, or Triton™ X-100: a. Add 100 myl of -20°C 90% methanol to each well and incubate for 5 minutes at RT. OR b. Add 100 myl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.

Application Details

	4. Remove the permeabilization buffer, and wash the wells twice with 100 myl of 1× PBS. 5. Remove the PBS, and block the cells by adding 100 myl of to each well. Incubate for 30 minutes at RT. 6. Remove the blocking buffer and add 50 myl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT. 7. Remove the primary antibody, and wash the wells three times with 100 myl of 1× PBS. 8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 myl to each well, and incubate in the dark for 1 hour at RT. 9. Remove the second step reagent, and wash the wells three times with 100 myl of 1× PBS. 10. Remove the PBS, and counter-stain the nuclei by adding 200 myl per well of 2 myg/ml Hoechst 33342 in 1× PBS to each well at least 15 minutes before imaging. 11. View and analyze the cells on an appropriate imaging instrument.
Comment:	Related Products: ABIN968537, ABIN967389
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	250 µg/mL
Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C
Storage Comment:	Store undiluted at -20°C.

Publications

Product cited in:	Naga Prasad, Laporte, Chamberlain, Caron, Barak, Rockman: "Phosphoinositide 3-kinase regulates beta2-adrenergic receptor endocytosis by AP-2 recruitment to the receptor/beta-arrestin complex." in: **The Journal of cell biology**, Vol. 158, Issue 3, pp. 563-75, (2002) (PubMed). Ros-Baro, Lopez-Iglesias, Peiro, Bellido, Palacin, Zorzano, Camps: "Lipid rafts are required for

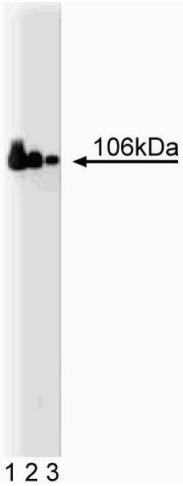
Publications

GLUT4 internalization in adipose cells." in: **Proceedings of the National Academy of Sciences of the United States of America**, Vol. 98, Issue 21, pp. 12050-5, (2001) (PubMed).

Laporte, Oakley, Zhang, Holt, Ferguson, Caron, Barak: "The beta2-adrenergic receptor/betaarrestin complex recruits the clathrin adaptor AP-2 during endocytosis." in: **Proceedings of the National Academy of Sciences of the United States of America**, Vol. 96, Issue 7, pp. 3712-7, (1999) (PubMed).

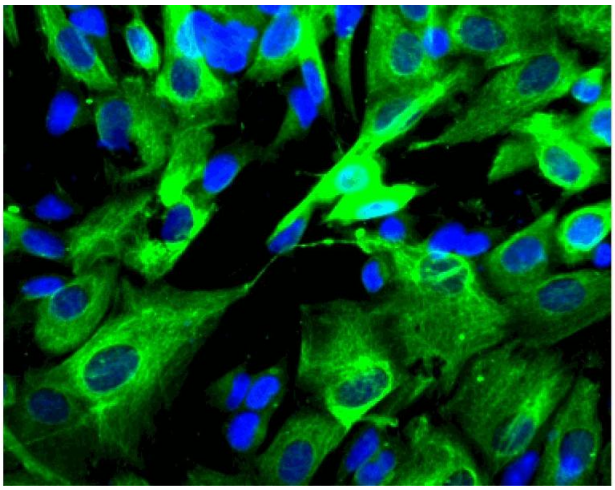
Ponnambalam, Robinson, Jackson, Peiperl, Parham: "Conservation and diversity in families of coated vesicle adaptins." in: **The Journal of biological chemistry**, Vol. 265, Issue 9, pp. 4814-20, (1990) (PubMed).

Images



Western Blotting

Image 1. Western blot analysis of Adaptin beta on a Jurkat cell lysate (Human T-cell leukemia, ATCC TIB-152) (left). Lane 1: 1:5000, lane 2: 1:10,000, lane 3: 1:20,000 dilution of the mouse anti-Adaptin beta antibody.



Immunofluorescence

Image 2. Immunofluorescent staining of SK-N-SH cells (Human neuroblastoma, ATCC HTB-11) (right). Cells were seeded in a collagen coated 384-well imaging plate at ~ 8,000 cells per well. After overnight incubation, cells were stained using the Triton-X 100 fix/perm protocol and the mouse anti-Adaptin beta antibody. The second step reagent was Alexa Fluor® 488 goat anti-mouse Ig (Invitrogen). The image was taken on a BD Pathway™ 855 or 435 Bioimager using a 20x objective. This antibody also stained SH-SY5Y (Human neuroblastoma, ATCC CRL-2266), C6 (Rat glioma,

ATCC CCL-107), U-87 MG (Human glioblastoma cells, ATCC HTB-14) and U-373 cells (Human glioblastoma cells, ATCC HTB-17, discontinued) using both the Triton-X 100 and methanol fix/perm protocols.