Technical Data Sheet

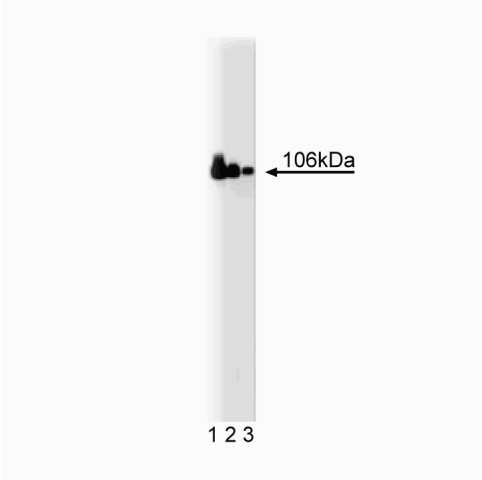
Purified Mouse Anti-Adaptin β

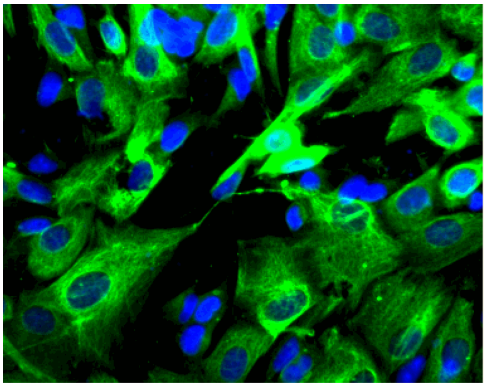
Product Information

Material Number:	**610381**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	74/Adaptin β
Immunogen:	Human Adaptin β aa. 75-245
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Mouse, Rat, Dog, Chicken, Frog
Target MW:	106 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

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 (γ, α, δ, or ε and β1, β2, β3, or β4, respectively) that are noncovalently linked to one medium chain (µ1, µ2, µ3, or µ4) and one small chain (σ1, σ2, σ3, or σ4). 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. β Adaptin subunits (β1, β2, β3, β4) lack sequence homology to adaptins α, γ, δ, and ε, but all of these subunits share a similar domain structure. Adaptin β1 (also known as Adaptin β') and β2 (also known as Adaptin β) have 83% amino acid identity and are found in the AP1 and AP2 complexes, respectively.





Western blot analysis of Adaptin β 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 β antibody.*

Immunofluorescent staining of SK-N-SH cells (Human neuroblastoma; ATCC HTB-11) (right). *Cells were seeded in a collagen coated 384-well imaging plate (Material # 353962) at ~ 8,000 cells per well. After overnight incubation, cells were stained using the Triton-X 100 fix/perm protocol (see Recommended Assay Procedure) and the mouse anti-Adaptin β 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, investigators may refer to: http://www.atcc.org/MisidentifiedCellLines/tabid/683/Default.aspx) using both the Triton-X 100 and methanol fix/perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Application Notes

Application

Western blot	Routinely Tested
Immunoprecipitation	Tested During Development
Immunohistochemistry	Tested During Development
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl 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 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl 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 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/monoclonal_anti.jsp

Suggested Companion Products

Catalog Number	Name	Size	Clone
611451	Jurkat Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
353962	BD Falcon™ 384-well Imaging Plate	NA	test clone
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)

Product Notices

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 deposits in plumbing.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Kirchhausen T, Nathanson KL, Matsui W. Structural and functional division into two domains of the large (100- to 115-kDa) chains of the clathrin-associated protein complex AP-2. *Proc Natl Acad Sci U S A.* 1989; 86(8):2612-2616. (Biology)

Laporte SA, Oakley RH, Zhang J. The beta2-adrenergic receptor/betaarrestin complex recruits the clathrin adaptor AP-2 during endocytosis. *Proc Natl Acad Sci U S A.* 1999; 96(7):3712-3717. (Biology: Immunofluorescence)

Naga Prasad SV, Laporte SA, Chamberlain D, Caron MG, Barak L, Rockman HA. Phosphoinositide 3-kinase regulates beta2-adrenergic receptor endocytosis by AP-2 recruitment to the receptor/beta-arrestin complex. *J Cell Biol.* 2002; 158(3):563-575. (Biology: Western blot)

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Ros-Baro A, Lopez-Iglesias C, Peiro S. Lipid rafts are required for GLUT4 internalization in adipose cells. *Proc Natl Acad Sci U S A.* 2001; 98(21):12050-12055. (Biology: Western blot)