Technical Data Sheet

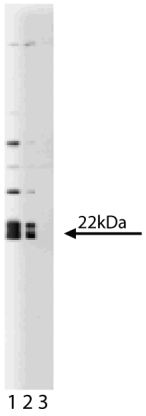
Purified Mouse Anti- Caveolin 1

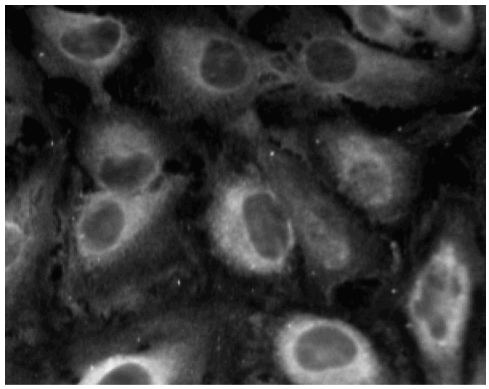
Product Information

Material Number:	**610057**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	C060
Immunogen:	Human Caveolin 1 aa. 1-97
Isotype:	Mouse IgM
Reactivity:	QC Testing: Human Tested in Development: Dog, Mouse, Rabbit, Rat
Target MW:	22 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Identified as a tyrosine phosphorylated protein in Rous sarcoma virus-transformed chick embryo fibroblasts (CEF), caveolin is now known to be ubiquitously expressed. Caveolin (also known as VIP21) localizes to non-clathrin membrane invaginations (caveolae) on the inner surface of the plasma membrane. This transmembrane protein plays a structural role in these specializations. Caveolin is also present at the trans-Golgi network (TGN) and similar quantities are found in apically and basolaterally destined transport vesicles. Caveolin is part of a complex containing glycosylphosphatidylinositol (GPI)-linked molecules and cytoplasmic signaling proteins. Caveolin is a transmembrane adaptor molecule that can simultaneously recognize GPI-linked proteins and interact with downstream cytoplasmic signaling molecules, such as c-yes, Annexin II, and hetero-trimeric G proteins. Caveolin-1 can generate two forms, α and ß, due to alternate splicing of the mRNA. Caveolin-1 forms large lipid-binding homo-oligomers which are believed to play a role in caveolae formation. It may also function as a scaffolding protein which concentrates and organizes signaling molecules, a role supported by the fact that caveolin-1 interacts directly with inactive Ras and G-protein α subunits.





Left: Western blot analysis of Caveolin 1 on a human endothelial lysate. *Lane 1: 1:500, lane 2: 1:1000, lane 3: 1:2000 dilution of the Caveolin 1 antibody.* ***Right: Immunofluorescent staining of HeLa (ATCC CCL-2) cells.*** *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~ 10 000 cells per well. After overnight incubation, cells were stained using the Triton™ X-100 perm protocol and the anti-Caveolin 1 antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). Images were taken on a BD Pathway™ 855 bioimaging system using a 20x objective. This antibody also stained A549 (ATCC CCL-185) cells and worked with both the Triton™ X-100 and alcohol fix/perm protocols (see Recommended Assay Procedure). This antibody is not recommended for staining U-2 OS cells.*

Preparation and Storage

Store undiluted at -20°C.

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

Application Notes

Application

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

BD Biosciences

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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
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611450	Human Endothelial Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(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. Triton is a trademark of the Dow Chemical Company.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. 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.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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Stan RV, Roberts WG, Predescu D, et al. Immunoisolation and partial characterization of endothelial plasmalemmal vesicles (caveolae). *Mol Cell Biol.* 1997; 8(4):595-605. (Biology: Electron microscopy)