Datasheet for ABIN967797

anti-GNB2L1 antibody (AA 113-317)

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Overview

Quantity:	150 µg
Target:	GNB2L1
Binding Specificity:	AA 113-317
Reactivity:	Human, Mouse, Rat, Cow, Dog, Chicken, Frog
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This GNB2L1 antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (IHC), Immunoprecipitation (IP), BioImaging (BI)

Product Details

Immunogen:	Rat RACK1 aa 113-317
Clone:	20-RACK1
Isotype:	IgM
Cross-Reactivity:	Human, Cow (Bovine), Chicken, Dog (Canine), Frog, Mouse (Murine)
Characteristics:	1. Since applications vary, each investigator should titrate the reagent to obtain optimal results. 2. Please refer to us for technical protocols. 3. 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. 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States. 6. Triton is a trademark of the Dow Chemical Company.
Purification:	The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Target Details

Target:	GNB2L1
Alternative Name:	RACK1 (GNB2L1 Products)
Background:	Several proteins which specifically bind to PKC have been classified as RACKs (receptors for activated C-kinase). RACK1 was cloned from a rat brain cDNA expression library by screening for proteins that bind PKC in the presence of phosphatidylserine, diacylglycerol, and calcium in a PKC overlay assay. By sequence homology, RACK1 appears to belong to a superfamily that includes the ß subunit of G proteins. All of these proteins contain five to eight internal repeat elements known as WD40 motifs, which appear to have a role in protein-protein interactions. In addition, RACK1 contains two short sequences homologous to a PKC-binding sequence identified in Annexin I and in the brain PKC inhibitor KCIP. The binding of RACK1 to PKC is dose-dependent and occurs at a site on PKC that is distinct from the catalytic domain, indicating that RACK1 is not a PKC substrate.
Molecular Weight:	36 kDa
Pathways:	cAMP Metabolic Process, Positive Regulation of Endopeptidase Activity

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. 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

Application Details

	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
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:	Birikh, Sklan, Shoham, Soreq: "Interaction of "readthrough" acetylcholinesterase with RACK1 and PKCbeta II correlates with intensified fear-induced conflict behavior." in: **Proceedings of the National Academy of Sciences of the United States of America**, Vol. 100, Issue 1, pp. 283-8, (2003) (PubMed). Liedtke, Yun, Kyle, Wang: "Protein kinase C epsilon-dependent regulation of cystic fibrosis transmembrane regulator involves binding to a receptor for activated C kinase (RACK1) and RACK1 binding to Na+/H+ exchange regulatory factor." in: **The Journal of biological chemistry**,

Publications

Vol. 277, Issue 25, pp. 22925-33, (2002) (PubMed).

Chang, Chiang, Cartwright: "The interaction of Src and RACK1 is enhanced by activation of protein kinase C and tyrosine phosphorylation of RACK1." in: **The Journal of biological chemistry**, Vol. 276, Issue 23, pp. 20346-56, (2001) (PubMed).

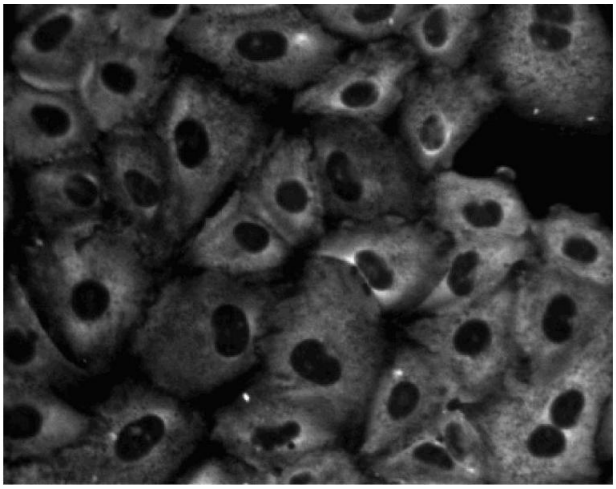
Smart, Ying, Anderson: "Hormonal regulation of caveolae internalization." in: **The Journal of cell biology**, Vol. 131, Issue 4, pp. 929-38, (1996) (PubMed).

Ron, Chen, Caldwell, Jamieson, Orr, Mochly-Rosen: "Cloning of an intracellular receptor for protein kinase C: a homolog of the beta subunit of G proteins." in: **Proceedings of the National Academy of Sciences of the United States of America**, Vol. 91, Issue 3, pp. 839-43, (1994) (PubMed).

Images

Western Blotting

Image 1. Western blot analysis of RACK1 on a Jurkat lysate. Lane 1: 1:2500, lane 2: 1:5000, lane 3: 1:10000 dilution of the RACK1 antibody.



Immunofluorescence

Image 2. Immunofluorescent staining of A549 (ATCC CCL-185) cells. Cells were seeded in a 96 well imaging plate at ~ 10 000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the anti-RACK1 antibody. The second step reagent was FITC goat anti mouse Ig. The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained U-2 OS (ATCC HTB-96) and HeLa (ATCC CCL-2) cells using

both the Triton™ X-100 and alcohol perm protocols.

Image 3.