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anti-GNB2L1 antibody (AA 113-317)

2 Images



Publications

Rat RACK1 aa 113-317



Go to Product page

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| Quantity: | 50 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:

| Clone: | 20-RACK1 |
|-------------------|--|
| Isotype: | IgM |
| Cross-Reactivity: | Human, Cow (Bovine), Chicken, Dog (Canine), Frog, Mouse (Murine) |
| Characteristics: | Since applications vary, each investigator should titrate the reagent to obtain optimal results. 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.
- 7. All other brands are trademarks of their respective owners.

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 | |

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 dosedependent 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 \sim 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 1x 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 1x 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 1x PBS.
- 10. Remove the PBS, and counter-stain the nuclei by adding 200 myl per well of 2 myg/ml Hoechst 33342 in $1\times$ 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

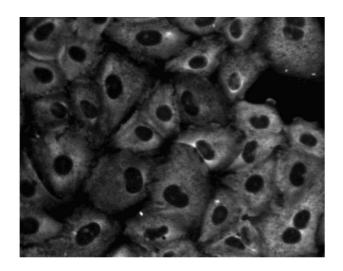
RACK1 binding to Na+/H+ exchange regulatory factor." in: **The Journal of biological chemistry**, Vol. 277, Issue 25, pp. 22925-33, (2002) (PubMed).

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).

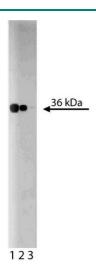
Ron, Chen, Caldwell, Jamieson, Orr, Mochly-Rosen: "Cloning of an intracellular receptor for protein kinase C: a homolog of the beta subunit of G proteins." in: **Proceedings of the National Academy of Sciences of the United States of America**, Vol. 91, Issue 3, pp. 839-43, (1994) (PubMed).

Images



Immunofluorescence

Image 1. 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.



Western Blotting

Image 2. 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.