Technical Data Sheet

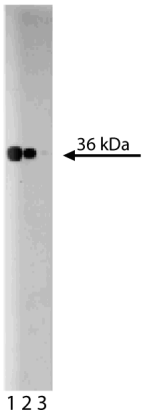
Purified Mouse Anti-RACK1

Product Information

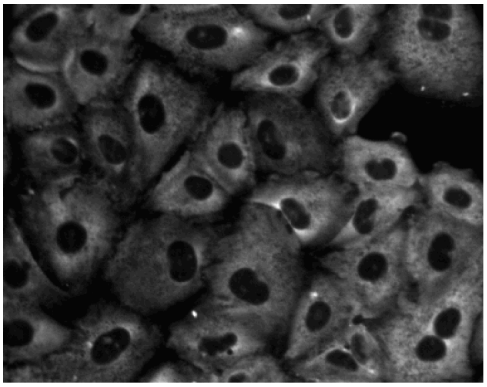
Material Number:	**610177**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	20/RACK1
Immunogen:	Rat RACK1 aa 113-317
Isotype:	Mouse IgM
Reactivity:	QC Testing: Human Tested in Development: Bovine, Chicken, Dog, Frog, Mouse, Rat
Target MW:	36 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Several proteins which specifically bind to PKC have been classified as RACKs (**r**eceptors for **a**ctivated **C**-**k**inase). 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.



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.*



Immunofluorescent staining of A549 (ATCC CCL-185) 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 alcohol perm protocol and the anti-RACK1 antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). 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 (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
Bioimaging	Tested During Development
Immunohistochemistry	Tested During Development
Immunoprecipitation	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.

For more detailed information on Bioimaging and Western Blot applications, please refer to http://www.bdbiosciences.com/support/resources/cell_biology/index.jsp

Suggested Companion Products

Catalog Number	Name	Size	Clone
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
611451	Jurkat 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)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols 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 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.

References

Birikh KR, Sklan EH, Shoham S, Soreq H. Interaction of "readthrough" acetylcholinesterase with RACK1 and PKCbeta II correlates with intensified fear-induced conflict behavior. *Proc Natl Acad Sci U S A.* 2003; 100(1):283-288. (Clone-specific: Immunohistochemistry, Western blot)

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