Datasheet for ABIN967703

anti-Crk antibody (AA 102-304)

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Overview

Quantity:	150 µg
Target:	Crk (CRK)
Binding Specificity:	AA 102-304
Reactivity:	Human, Mouse, Rat, Dog, Cow, Chicken, Frog
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This Crk antibody is un-conjugated
Application:	Western Blotting (WB), BioImaging (BI), Immunoprecipitation (IP)

Product Details

Immunogen:	Human Crk aa. 102-304
Clone:	22-Crk
Isotype:	IgG2a
Cross-Reactivity:	Cow (Bovine), Chicken, Dog (Canine), Frog, Mouse (Murine), Rat (Rattus)
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:	Crk (CRK)
Alternative Name:	Crk (CRK Products)
Background:	Crk was first isolated as the v-crk oncogene from chicken retroviruses CT10 and ASV-1. All human cell lines examined to date express a 40 kDa Crk protein. In addition, there is variable expression of 42 kDa and 28 kDa Crk proteins. The c-crk gene is one of a class of genes, such as Nck and GRB2/ASH, which encode proteins that consist mainly of SH2 and SH3 domains. These proteins function as adaptor molecules in tyrosine kinase signal transduction pathways. The SH2 domains interact with phosphotyrosine-containing peptides, while the SH3 domains can enhance this interaction and/or bind to other cellular components. Both the SH2 and SH3 domains of the human Crk protein are required for differentiation of PC12 cells. Thus, Crk has a role in an NGF-induced signaling pathway that involves activation of p21ras. Furthermore, three proteins of 118 kDa, 125 kDa, and 136 kDa which specifically bind to the Crk SH3 domain have been identified.
Molecular Weight:	40 kDa
Pathways:	Neurotrophin Signaling Pathway, CXCR4-mediated Signaling Events, Signaling of Hepatocyte Growth Factor Receptor

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.

Application Details

	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 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: ABIN967389, ABIN968535
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:	Miller, Chen, Gharib, Wang, Thomas, Misek, Giordano, Yee, Orringer, Hanash, Beer: "Increased C-CRK proto-oncogene expression is associated with an aggressive phenotype in lung adenocarcinomas." in: **Oncogene**, Vol. 22, Issue 39, pp. 7950-7, (2003) (PubMed). Cho, Klemke: "Purification of pseudopodia from polarized cells reveals redistribution and activation of Rac through assembly of a CAS/Crk scaffold." in: **The Journal of cell biology**, Vol.

156, Issue 4, pp. 725-36, (2002) (PubMed).

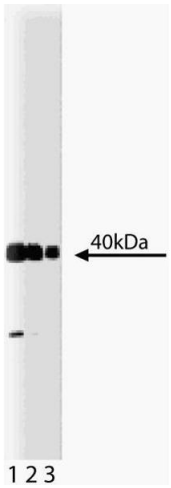
Liu, Kimura, Baumann, Saltiel: "APS facilitates c-Cbl tyrosine phosphorylation and GLUT4 translocation in response to insulin in 3T3-L1 adipocytes." in: **Molecular and cellular biology**, Vol. 22, Issue 11, pp. 3599-609, (2002) (PubMed).

Smith, Richardson, Kopf, Yoshida, Hollingsworth, Kornbluth: "Apoptotic regulation by the Crk adapter protein mediated by interactions with Wee1 and Crm1/exportin." in: **Molecular and cellular biology**, Vol. 22, Issue 5, pp. 1412-23, (2002) (PubMed).

Girardin, Yaniv: "A direct interaction between JNK1 and CrkII is critical for Rac1-induced JNK activation." in: **The EMBO journal**, Vol. 20, Issue 13, pp. 3437-46, (2001) (PubMed).

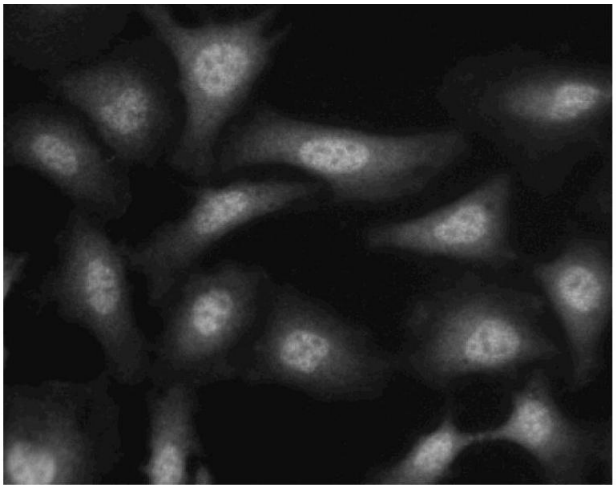
There are more publications referencing this product on: Product page

Images



Western Blotting

Image 1. Western blot analysis of Crk on a HeLa lysate. Lane 1: 1:5000, lane 2: 1:10000, lane 3: 1:20000 dilution of the Crk antibody.



Immunofluorescence

Image 2. Immunofluorescent staining of HeLa (ATCC CCL-2) cells. Cells were seeded in a 96 well imaging plate at ~ 10 000 cells per well. After overnight incubation, cells were stained using the Triton™ X-100 perm protocol and the anti-Crk antibody. The second step reagent was FITC goat anti mouse Ig. Images were taken on a BD Pathway 855 Bioimager system using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96)

cells and worked with both the Triton™ X/100 and alcohol perm protocols.

Image 3.