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Datasheet for 600-401-998

Rock-2 phospho Y256 Antibody

Overview

Description:	Anti-ROCK2 pY256 (RABBIT) Antibody - 600-401-998
Item No.:	600-401-998
Size:	100 µg
Applications:	ELISA, WB
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	This antibody is designed, produced, and validated as part of a collaboration between Rockland and the National Cancer Institute (NCI) and is suitable for Cancer, Immunology and Nuclear Signaling research. Progression of tumors to invasive and metastatic forms requires that tumor cells undergo dramatic morphological changes, a process regulated by Rho GTPases. Increased levels of RhoA or RhoC, as well as the Rho effector proteins ROCK-1 or ROCK-2, were observed in various human carcinomas. In vivo studies have also shown that ROCK inhibition reduced the invasiveness of several tumor cell lines. It is suggested that Rho and ROCK signaling contribute to the morphological changes and metastatic behavior of some tumor cells. Thus, elevated Rho and ROCK expression is associated with tumorigenesis, particularly during the progression to invasive and metastatic phenotypes. Phosphorylation of certain residues in ROCK-2 may be critical for its kinase activity.
Synonyms:	rabbit anti-ROCK2 antibody, Rho-associated protein kinase 2, Rho-associated coiled-coil-containing protein kinase 2, p164 ROCK-2, Rho kinase 2, Rho-associated coiled-coil-containing protein kinase II, ROCK-II, ROCK-2, ROCK 2
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	ROCK2
Reactivity:	Human

PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic phospho-peptide corresponding to residues surrounding Y256 of human ROCK-2.
Purity/Specificity:	This product was affinity purified from monospecific antiserum by immunoaffinity chromatography using phospho-peptide coupled to agarose beads followed by solid phase adsorption against nonphospho-peptide. This antibody is specific for human ROCK-2 protein phosphorylated at Y256. A BLAST analysis was used to suggest cross-reactivity with ROCK-2 from human, mouse, rat, chicken, dog, bovine, Xenopus and Drosophila based on a 100% homology with the immunizing sequence. Cross-reactivity with ROCK-2 from other sources has not been determined.
Relevant Links:	• UniProtKB - O75116 • NCBI - 4520225 • GeneID - 9475

Application Details

Tested Applications:	ELISA, WB
Application Note:	This affinity purified antibody has been tested for use in ELISA and western blotting. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 164 kDa in size corresponding to phosphorylated ROCK-2 by western blotting in the appropriate cell lysate or extract. This phospho-specific polyclonal antibody reacts with human ROCK-2 pY256 and shows minimal reactivity by ELISA against the non-phosphorylated form of the immunizing peptide.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:2,000 - 1:20,000
IHC:	User Optimized
WB:	1:200 - 1:2,000

Formulation

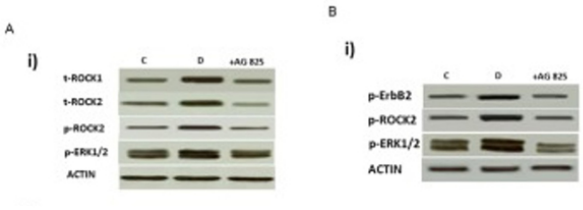
Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

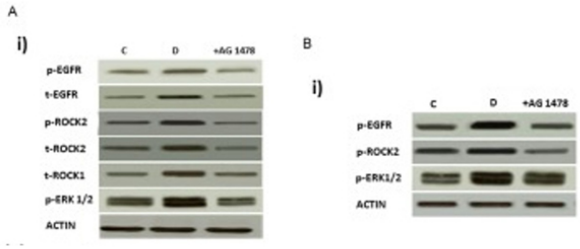
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

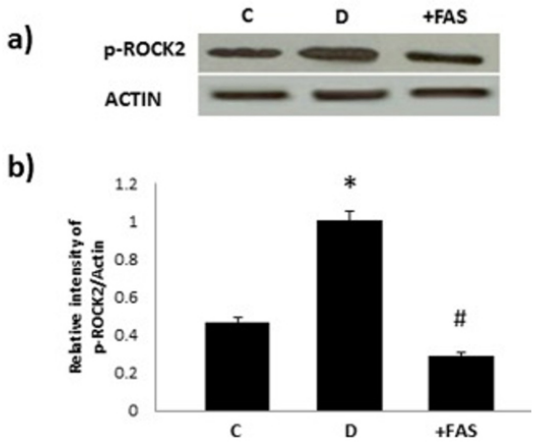
Images



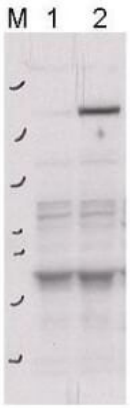
Western Blot
A-B) The effect of chronic (A) or acute (B) treatment with AG825 on ROCKs and ERK1/2 signaling in the isolated mesenteric bed of diabetic rats.
Panel i) is a representative Western blot showing total (t−) or phosphorylated (p−) levels of the stated proteins in the isolated mesenteric bed from normal controls (C), diabetic (D) and diabetic animals treated either chronically (1 mg/kg/alt-diem) for 4 weeks (shown in figure 3A) or acutely (10−5M) (shown in 3B) with AG825 (+AG825). Fig 3.
PMID: 23826343



Western Blot
A-B) Chronic (A) or acute (B) treatment with AG1478, a selective inhibitor of EGFR, attenuates diabetes-induced elevation in ROCK and ERK1/2 signaling in the mesenteric vascular bed.
A) Panel i) is a representative western blot showing total (t−) or phosphorylated (p−) levels of the stated proteins in the isolated mesenteric bed from normal controls (C), diabetic (D) and diabetic animals treated for 4 weeks with AG1478 (1 mg/kg/alt-diem; +AG825).
B) Panel i) is a representative western blot showing phosphorylated (p−) levels of the stated proteins in the isolated mesenteric bed from normal controls (C), diabetic (D) and diabetic animals treated for 4 weeks with AG1478 (1 mg/kg/alt-diem; +AG825). Fig 5. PMID: 23826343



Western Blot
Panel a) is representative Western blot showing phosphorylated (p-) levels of the stated proteins in the isolated mesenteric bed from normal controls (C), diabetic (D) and diabetic mesenteric bed treated with fasudil (10−6M;+FAS). Panel b) is densitometry histogram showing phosphorylated (p-) levels of the stated proteins normalized to actin. N = 5; Mean±SD. Asterisk (*) indicates significantly different ($p<0.05$) mean values from normal non-diabetic rats (C) whereas hash (#) indicates significantly different mean values ($p<0.05$) from diabetic rats (D). Fig 6.
PMID: 23826343



Western Blot
Western blot using Rockland's affinity purified anti-ROCK-2 pY256 antibody shows detection of phosphorylated ROCK-2 in transfected 293T cells. Lane 1 shows lysate expressing exogenous ROCK-2 where the insert contains the transversion Y256A. Lane 2 shows the lysate expressing WT ROCK-2. A lysate containing a GST null vector also shows no specific staining (data not shown). Primary antibody was used at a 1:1000 dilution. Personal Communication, A. Papageorge and X. Qian, NCI, Bethesda, MD.

References

- Akhtar S et al. Activation of ErbB2 and downstream signalling via Rho kinases and ERK1/2 contributes to diabetes-induced vascular dysfunction. *PLoS One.* (2013)
- Herhaus L. et al. OTUB1 enhances TGFβ signalling by inhibiting the ubiquitylation and degradation of active SMAD2/3. *Nat Commun.* (2013)

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.