Product Datasheet

ROCK1 Antibody
NB110-57465

Unit Size: 0.1 ml

Store at -20C. Avoid freeze-thaw cycles.

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NB110-57465

ROCK1 Antibody (EP786Y)

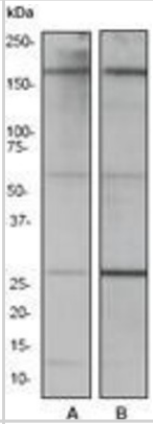
Product Information	
Unit Size	0.1 ml
Concentration	This product is unpurified. The exact concentration of antibody is not quantifiable.
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	EP786Y
Preservative	0.01% Sodium Azide
Isotype	IgG
Purity	Tissue culture supernatant
Buffer	PBS 49%, Glycerol 50%, BSA 0.05%
Target Molecular Weight	158 kDa

Product Description	
Host	Rabbit
Gene ID	6093
Gene Symbol	ROCK1
Species	Human, Mouse, Rat, Bovine
Specificity/Sensitivity	This antibody recognizes both the cleaved C-terminus of ROCK-1 (30 kDa) and full length protein.
Immunogen	A synthetic peptide corresponding to residues near C-terminus of human ROCK1 was used as an immunogen.
Notes	Produced using Abcam's RabMab® technology. RabMab® technology is covered by the following U.S. Patents, No. 5,675,063 and/or 7,429,487.

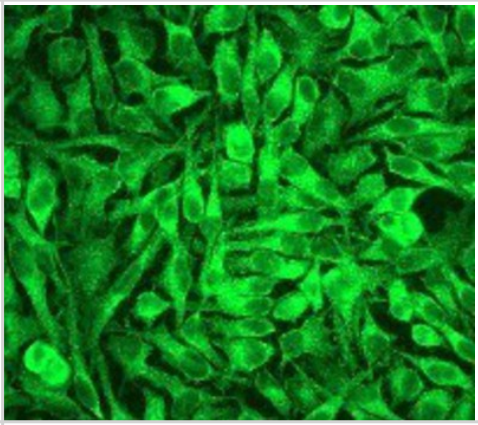
Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Flow Cytometry 1:1000, Immunocytochemistry/Immunofluorescence 1:250-500, Immunohistochemistry 1:10-1:500, Immunohistochemistry-Frozen 1:100, Immunohistochemistry-Paraffin 1:50-100, Immunoprecipitation 1:50, Western Blot 1:1000-10000
Application Notes	In Western blot this antibody detects a bands at approximately 30 & 160 kDa, corresponding to both C-terminal cleaved fragment and full length ROCK1. Immunohidtochemistry Frozen reported in literature (PMID: 19295659)

Images

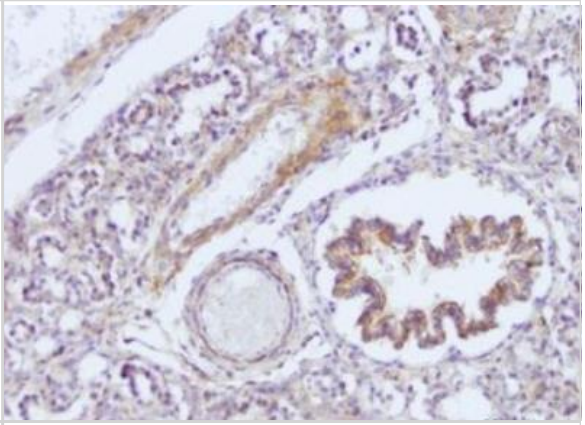
Western Blot: ROCK1 Antibody (EP786Y) [NB110-57465] Untreated HeLa cell lysate (A) and camptothecin treated HeLa lysate (B).



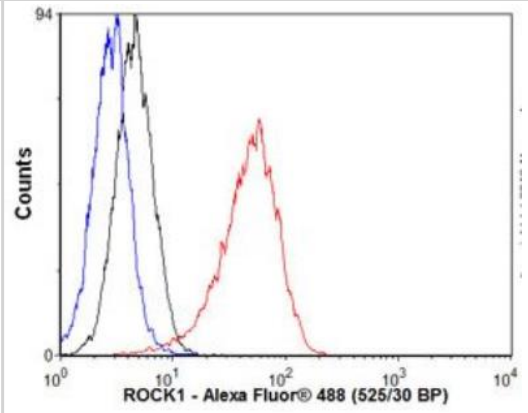
Immunocytochemistry/Immunofluorescence: ROCK1 Antibody (EP786Y) [NB110-57465] - HeLa cells.



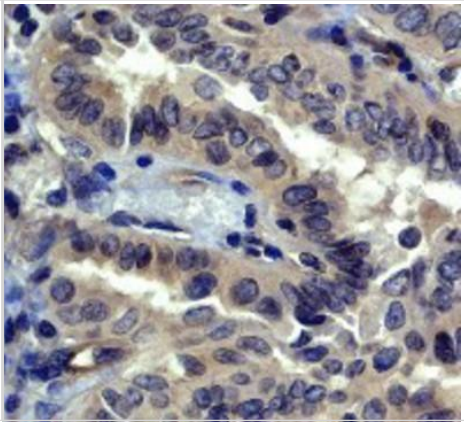
Immunohistochemistry-Paraffin: ROCK1 Antibody (EP786Y) [NB110-57465] - Mouse embyro 18dpc tissue.



Flow Cytometry: ROCK1 Antibody (EP786Y) [NB110-57465] - Overlay histogram showing HeLa cells (red line).



Immunohistochemistry-Paraffin: ROCK1 Antibody (EP786Y) [NB110-57465] - Immunohistochemical analysis of paraffin-embedded human thyroid gland carcinoma using anti-ROCK1 (cat # 110-57465).



Publications

Li HP, Huang HY, Lai YR et al. Silencing of miRNA-148a by hypermethylation activates the integrin-mediated signaling pathway in nasopharyngeal carcinoma. Oncotarget. 2014 Sep 15 [PMID: 25277193] (WB, Human)

Details:
ROCK1 antibody used for WB on lysates of miR-148a transient transfected TW02 cells (Figure 4b), shROCK1 knockdown TW02 and TW06 cells (Figure 5a), NPC tumor (Figure 5f),

Zhou X, Wei M, Wang W et al. MicroRNA-340 suppresses osteosarcoma tumor growth and metastasis by directly targeting ROCK1. Biochem Biophys Res Commun 2013 Aug 9 [PMID: 23872151] (WB, Human)

Li J, Song Y, Wang Y, Luo J, Yu Wet al. MicroRNA-148a suppresses epithelial-to-mesenchymal transition by targeting ROCK1 in non-small cell lung cancer cells. Mol Cell Biochem 2013 May 14 [PMID: 23670799] (WB, Human)

Zucchini-Pascal N, de Sousa G, Pizzol J et al. Pregnane X receptor activation protects rat hepatocytes against deoxycholic acid-induced apoptosis. Liver Int. 2010 Feb. [PMID: 19737350]

Zheng B, Liang L, Wang C et al. MicroRNA-148a Suppresses Tumor Cell Invasion and Metastasis by Downregulating ROCK1 in Gastric Cancer. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research. 2011 Oct 12. [PMID: 21994419]

Procedures

Immunohistochemistry Protocol for ROCK1 Antibody (NB110-57465)
Immunohistochemistry Protocol for Paraffin-embedded Tissues

1. Solutions and reagents
1.1. Xylene
1.2. Ethanol, anhydrous denatured, histological grade (100%, 95%, 70%)
1.3. Washing buffer:
TBST washing buffer: 1XTBS/0.1% Tween-20
To prepare stock solution of 10X TBS: add 24.2 g Trizma base and 80 g sodium chloride to 1L of dH2O. Adjust pH to 7.6.
Working solution. 1XTBST/0.1% Tween-20: add 100ml 10XTBS to 900 ml dH2O. Add 1 ml Tween-20 and mix well.
1.4. Distilled water (dH2O)
1.5. Antigen Retrieval Solution:
0.01M Sodium Citrate Buffer, pH 6.0
To prepare stock solutions:
Solution A. 0.1 M citric acid solution: dissolve 21.0 g of citric acid, monohydrate (C6H8O7.H2O) in 1 liter of dH2O.
Solution B. 0.1M sodium citrate solution: dissolve 29.4 g trisodium citrate dihydrate (C6H5Na3O7.2H2O) in 1 liter of dH2O.
Working solution: Add 9 ml of Stock solution A and 41 ml of stock solution B to 450 ml of dH2O. Adjust pH to 6.0.
1.6. 3% Hydrogene Peroxide
1.7. Blocking buffer:
PBS (Dulbeccos Phosphate Buffered Salts, 1X, catalog #21-031-CV from Mediatech, Inc.) + 10% serum (serum origin depends on the host of the secondary antibody)
1.8. Hematoxylin QS (catalog #H-3404 from Vector Laboratories, Inc.)
1.9. Permanent Mounting medium (VectaMount, catalog# H-5000 Vector Laboratories, Inc.)

2. Protocol

2.1. Deparaffinization/Rehydration
2.1.1. Heat slides in an oven at 65C for 1 hour.
2.1.2. De-paraffinize/hydrate using the following series of washes: two Xylene washes (5 min each), followed by two 100% ethanol rinses (5 min each), followed by 95% ethanol, 70% ethanol, 50% ethanol, 30% ethanol, followed by H2O and a TBST wash for 5 min on a shaker.
2.2. Antigen Retrieval
2.2.1. Immerse slides into staining dish containing Antigen Retrieval Solution.
2.2.2. Place covered staining dish into the rice cooker. Add 120 ml of dH2O.
2.2.3. When cook is turned to warm (about 20 to 30 min), unplug the cooker and remove the staining dish to the bench top.
2.2.4. Allow to cool down, without cover, for 20 min.
2.3. Staining
2.3.1. Wash slides with TBST for 5 min on a shaker.
2.3.2. Inactivate endogenous peroxidase by covering tissue with 3% hydrogen peroxide for 10 min.
2.3.3. Wash slides three times with TBST (3 min each on a shaker).
2.3.4. Block slides with the blocking solution for 1 hour.
2.3.5. Dilute primary antibody in the blocking buffer per recommendation on the data sheet.
2.3.6. Apply primary antibody to each section and incubate overnight in the humidified chamber (4C).
2.3.7. Wash slides three times with TBST (3 min each on a shaker).
2.3.8. Apply to each section secondary HRP-conjugated anti-rabbit antibody diluted in the blocking solution per manufacturers recommendation; incubate for 1 hour at room temperature.
2.3.9. Wash slides three times with TBST (3 min each on a shaker).
2.3.10. Add freshly prepared DAB substrate to the sections.
2.3.11. Incubate tissue sections with the substrate at room temperature until suitable staining develops (generally 2 to 5 min).
2.3.12. Rinse sections with water.
2.3.13. Counterstain with Hematoxylin.
2.3.14. Rinse sections with water.

2.3.15. Dehydrate samples using two rinses with 100% Ethanol (20 dips per rinse) followed by two rinses with Xylene (30 dips per rinse).
2.3.16. Mount coverslips on slides using Permount medium.

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Products Related to NB110-57465

NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
NB7160	Goat anti-Rabbit IgG Antibody [HRP]
NB810-56910-10mg	Rabbit IgG Isotype Control
H00006093-Q01-10ug	ROCK1 Partial Recombinant Protein

Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis.
Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our guarantee, please visit www.novusbio.com/guarantee.