Datasheet for ABIN733208

anti-RAF1 antibody (AA 31-130)

1 Validation | 2 Images | 1 Publication

Go to Product page

Overview

Quantity:	100 µL
Target:	RAF1
Binding Specificity:	AA 31-130
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This RAF1 antibody is un-conjugated
Application:	ELISA, Immunofluorescence (Cultured Cells) (IF (cc)), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)), Immunofluorescence (Paraffin-embedded Sections) (IF (p)), Immunohistochemistry (Frozen Sections) (IHC (fro))

Product Details

Immunogen:	KLH conjugated synthetic peptide derived from human c Raf
Isotype:	IgG
Cross-Reactivity:	Human
Predicted Reactivity:	Mouse,Rat,Dog,Cow,Horse,Rabbit
Purification:	Purified by Protein A.

Target Details

Target:	RAF1

Target Details

Alternative Name:	c-Raf/Raf1 (RAF1 Products)
Background:	Synonyms: NS5, CRAF, Raf-1, c-Raf, CMD1NN, RAF proto-oncogene serine/threonine-protein kinase, Proto-oncogene c-RAF, RAF1, RAF Background: Serine/threonine-protein kinase that acts as a regulatory link between the membrane-associated Ras GTPases and the MAPK/ERK cascade, and this critical regulatory link functions as a switch determining cell fate decisions including proliferation, differentiation, apoptosis, survival and oncogenic transformation. RAF1 activation initiates a mitogen-activated protein kinase (MAPK) cascade that comprises a sequential phosphorylation of the dual-specific MAPK kinases (MAP2K1/MEK1 and MAP2K2/MEK2) and the extracellular signal-regulated kinases (MAPK3/ERK1 and MAPK1/ERK2). The phosphorylated form of RAF1 (on residues Ser-338 and Ser-339, by PAK1) phosphorylates BAD/Bcl2-antagonist of cell death at 'Ser-75'. Phosphorylates adenylyl cyclases: ADCY2, ADCY5 and ADCY6, resulting in their activation. Phosphorylates PPP1R12A resulting in inhibition of the phosphatase activity. Phosphorylates TNNT2/cardiac muscle troponin T. Can promote NF-kB activation and inhibit signal transducers involved in motility (ROCK2), apoptosis (MAP3K5/ASK1 and STK3/MST2), proliferation and angiogenesis (RB1). Can protect cells from apoptosis also by translocating to the mitochondria where it binds BCL2 and displaces BAD/Bcl2-antagonist of cell death. Regulates Rho signaling and migration, and is required for normal wound healing. Plays a role in the oncogenic transformation of epithelial cells via repression of the TJ protein, occludin (OCLN) by inducing the up-regulation of a transcriptional repressor SNAI2/SLUG, which induces down-regulation of OCLN. Restricts caspase activation in response to selected stimuli, notably Fas stimulation, pathogen-mediated macrophage apoptosis, and erythroid differentiation.
Gene ID:	5894
UniProt:	P04049
Pathways:	MAPK Signaling, RTK Signaling, Fc-epsilon Receptor Signaling Pathway, Neurotrophin Signaling Pathway, cAMP Metabolic Process, Stem Cell Maintenance, Hepatitis C, Autophagy, Signaling of Hepatocyte Growth Factor Receptor, VEGF Signaling, BCR Signaling

Application Details

Application Notes:	ELISA 1:500-1000
	IHC-P 1:200-400
	IHC-F 1:100-500
	IF(IHC-P) 1:50-200
	IF(IHC-F) 1:50-200

Application Details

	IF(ICC) 1:50-200
Restrictions:	For Research Use only

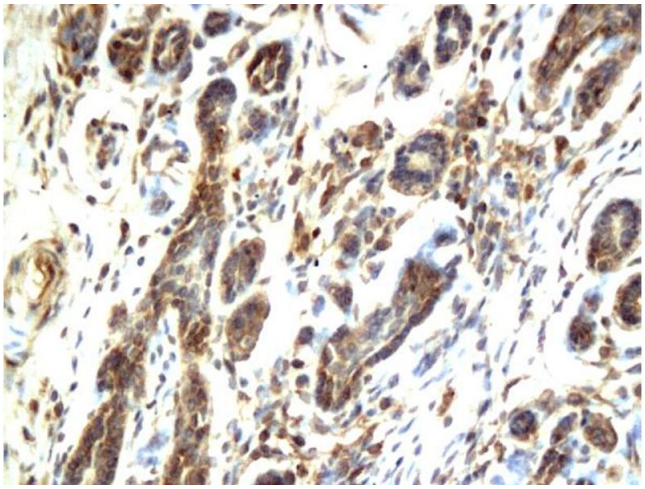
Handling

Format:	Liquid
Concentration:	1 μg/μL
Buffer:	0.01M TBS(pH 7.4) with 1 % BSA, 0.02 % Proclin300 and 50 % Glycerol.
Preservative:	ProClin
Precaution of Use:	This product contains ProClin: a POISONOUS AND HAZARDOUS SUBSTANCE, which should be handled by trained staff only.
Storage:	4 °C,-20 °C
Storage Comment:	Shipped at 4°C. Store at -20°C for one year. Avoid repeated freeze/thaw cycles.
Expiry Date:	12 months

Publications

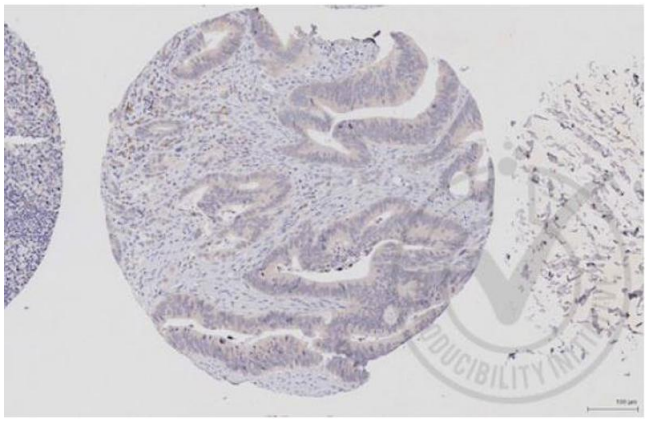
Product cited in:	Wang, Fu, Jiang, Yu, Wang, Han, Liu, Wang: "Bone marrow mononuclear cell transplantation promotes therapeutic angiogenesis via upregulation of the VEGF-VEGFR2 signaling pathway in a rat model of vascular dementia." in: **Behavioural brain research**, Vol. 265, pp. 171-80, (2014) (PubMed).

Images



Immunohistochemistry

Image 1. Formalin-fixed and paraffin embedded human breast cancer labeled with Anti-c-RafRaf1 Polyclonal Antibody, Unconjugated (ABIN733208) followed by conjugation to the secondary antibody and DAB staining



Immunohistochemistry

Image 2. Images provided the Independent Validation Program (badge number 29651)Formalin-fixed and paraffin embedded human colon labeled with Rabbit Anti-c-Raf/Raf1 Polyclonal Antibody (ABIN733208) at 1:250 overnight at room temperature followed by conjugation to secondary antibody.

Successfully validated (Immunohistochemistry (IHC))

by Immunohistochemistry Core, NYU Langone

Report Number: 029651

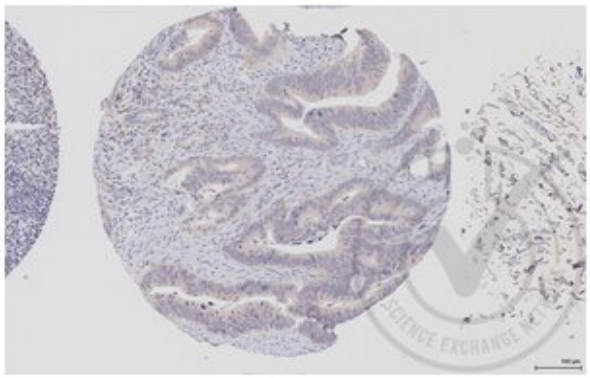
Date: Apr 01 2014

Lot Number:	130909
Method validated:	Immunohistochemistry (IHC)
Positive Control:	Human colon villus epithelial cells
Negative Control:	Human liver
Notes:	Raf1 exhibits uniformly low expression across all human tissues; higher expression found in colon, lower in liver. Tissue-specific expression is detected in colon, with expected low level expression in liver.
Primary Antibody:	- Antigen: V-Raf-1 Murine Leukemia Viral Oncogene Homolog 1 (RAF1) - Catalog number: ABIN733208 - Supplier: Bioss - Supplier catalog number: bs-1703R - Lot number: 130909
Secondary Antibody:	- Supplier: Ventana Medical Systems - Catalog number: 760-091 - Lot number: D07640BA
Isotype:	- Antibody: Rabbit IgG isotype control - Supplier: Ventana Medical Systems - Catalog number: 790-2014 - Lot number: C11245
Controls:	• Positive control: Human colon tissue stained with antibody • Negative control: Human liver tissue stained with antibody • Isotype control: Human colon tissue stained with isotype control • Secondary only control: Human colon tissue stained with secondary antibody only
Protocol:	• Immunohistochemistry was performed on a Ventana NEXes automated platform; instrument manufacturer specific reagents are italicized. • 1. Slides were preheated in convection oven at 60°C for 30 min • 2. Deparaffinization procedure: - 3 changes of Xylene, 5 min each - 3 changes of 100% Ethanol, 3 min each - 3 changes of 95% Ethanol, 3 min each - Rinsed in distilled water, 3 changes • 3. Heat retrieval procedure - Slides retrieved in 10.0 mM Citrate, pH6.0 in a 1000W microwave oven (~100°C) for 15 min. - Slides were allowed to cool (in citrate) for 30 min. - Slides were washed x 3 in Distilled water • 4. NEXes instrument procedure, iView DAB paraffin protocol (*abridged*): - Slide chamber warmed to 37°C • 5. Slides rinsed with *reaction buffer* x3

- 6. *iView Inhibitor (H2O2)* applied and incubated for 4 min
- 7. Slides rinsed with *reaction buffer*
- 8. Antibody Application - Primary antibody diluted 1:250 in PBS (100 microliter applied/slide) - Ventana Isotype control applied neat - Slides Incubated overnight at room temperature (~12 hours ~25°C)
- 9. Slides rinsed with *reaction buffer* x3
- 10. *iView Biotinylated IgG* applied and incubated for 8 min
- 11. Slides rinsed with *reaction buffer*
- 14. *iView Streptavidin-Horseradish Peroxidase* applied and incubated for 8 min
- 15. Slides rinsed with *reaction buffer*
- 16. *iView DAB/H2O2* applied and incubated for 8 min
- 17. Slides rinsed with *reaction buffer*
- 18. *iView Copper* applied and incubated for 4 min
- 19. Slides rinsed with *reaction buffer*
- 20. Slides washed in Dawn Detergent/tap water
- 21. Counterstain Procedure - Hematoxylin (Leica 560 MX) 30 sec - Slides washed in tap water, 1 min - Decolorized (10% Acetic Acid in 70% ethanol), 1 min - Slides washed in tap water, 1 min - Bluing (Austin Clear Ammonia), 1 min - Slides washed in tap water, 1 min
- 22. Dehydration/coverslipping procedure: - 3 changes of 95% Ethanol, 3 min each - 3 changes of 100% Ethanol, 3 min each - 3 changes of Xylene, 5 min each - Mounted with Permount
- 23. Imaging: Leica SCN 400F Whole Slide Scanner with Digital Image Hub and Leica Slidepath software

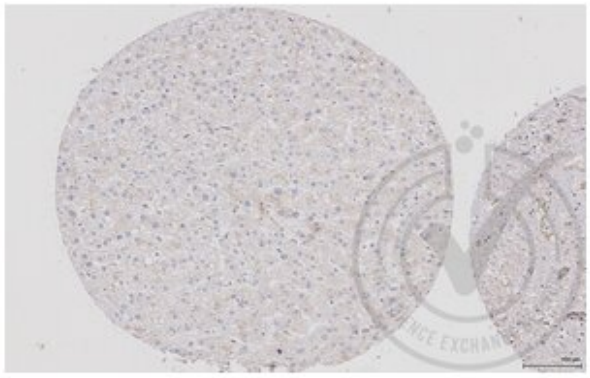
Experimental Notes:

- Deviations from protocol/procedure supplied by manufacturer:
- Step 1: Heated tissue 60°C for 30 minutes; manufacturer heats for 45 minutes.
- Step 2: No ethanol wash was performed during deparaffinization; manufacturer includes 1 wash of 80% ethanol for 3 minutes.
- Step 3.1: Slides were heated for 15 minutes; manufacturer provides a range of 15-20 minutes.
- Step 3.2: Slides were cooled for 30 minutes; manufacturer cools for 20 minutes.
- Step 4: Italicized reagents and incubation time are fixed instrument parameters.
- Step 5: Secondary species-specific serum block not used; manufacturer blocks with 5% normal goat serum for 2 hours.
- Step 8.1: Antibody diluted in PBS at 1:250; manufacture did not recommend diluent or dilution.
- Step 8.2.1: Primary antibody incubated at room temperature overnight; manufacturer incubates overnight 4°C with agitation.



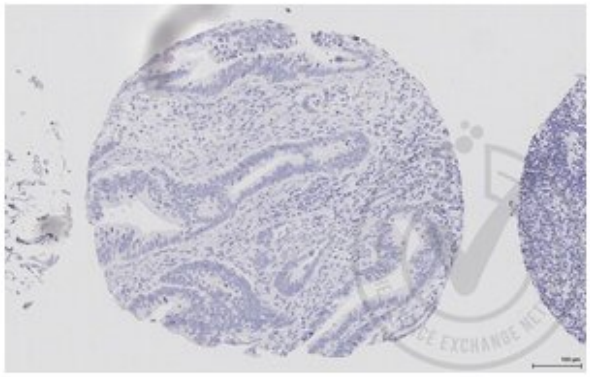
Validation image no. 1 for anti-V-Raf-1 Murine Leukemia Viral Oncogene Homolog 1 (RAF1) (AA 31-130) antibody (ABIN733208)

Figure 1: Human colon tissue stained with anti-RAF1 (brown) and counterstained with hematoxylin.



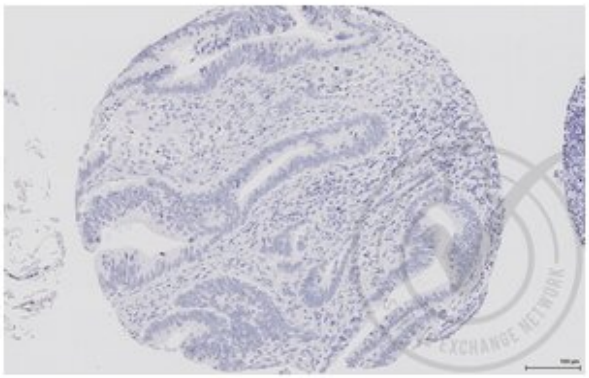
Validation image no. 2 for anti-V-Raf-1 Murine Leukemia Viral Oncogene Homolog 1 (RAF1) (AA 31-130) antibody (ABIN733208)

Figure 2: Human liver tissue stained with anti-RAF1 (brown) and counterstained with hematoxylin.



Validation image no. 3 for anti-V-Raf-1 Murine Leukemia Viral Oncogene Homolog 1 (RAF1) (AA 31-130) antibody (ABIN733208)

Figure 3: Human colon tissue stained with isotype control antibody (brown) and counterstained with hematoxylin.



Validation image no. 4 for anti-V-Raf-1 Murine Leukemia Viral Oncogene Homolog 1 (RAF1) (AA 31-130) antibody (ABIN733208)

Figure 4: Human colon tissue stained with secondary atnibody only (brown) and counterstained with hematoxylin.