

Datasheet for 600-401-MR3S**SMAD2 phospho S467 Antibody****Overview**

Description:	Anti-SMAD2 pS467 (RABBIT) Antibody - 600-401-MR3S
Item No.:	600-401-MR3S
Size:	25 µL
Applications:	Dot Blot, ELISA, IF, IHC
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	Smad2 (Mothers against decapentaplegic homolog 2) is a member of the Smad family of proteins which are similar to the gene products of the Drosophila gene 'mothers against decapentaplegic' (Mad) and the C. elegans gene Sma. SMAD proteins are signal transducers and transcriptional modulators that mediate multiple signaling pathways. This protein mediates the signal of the transforming growth factor (TGF)-beta, and thus regulates multiple cellular processes, such as cell proliferation, apoptosis, and differentiation. This protein is recruited to the TGF-beta receptors through its interaction with the SMAD anchor for receptor activation (SARA) protein. In response to TGF-beta signal, this protein is phosphorylated by the TGF-beta receptors. The phosphorylation induces the dissociation of this protein with SARA and the association with the family member SMAD4. The association with SMAD4 is important for the translocation of this protein into the nucleus, where it binds to target promoters and forms a transcription repressor complex with other cofactors. Anti-SMAD2 pS467 antibody is ideal for researchers interested in Cancer, Immunology and Nuclear Signaling research.
Synonyms:	Rabbit Anti-SMAD2 pS467 antibody, SMAD-2, SMAD 2, mothers against decapentaplegic homolog 2 antibody, MAD homolog 2, Mothers against DPP homolog 2, SMAD family member 2, MADH2, MADH 2, JV18-1
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	SMAD2
-------------------	-------

Reactivity:	Human
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	SMAD2 pS467 antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic phospho-peptide corresponding to a C-terminal of human SMAD2 protein.
Purity/Specificity:	Anti-SMAD2 pS467 antibody is directed against the phosphorylated form of human Smad2 protein at the pS467 residue. The product was affinity purified from monospecific antiserum by immunoaffinity purification. Antiserum was first purified against the phosphorylated form of the immunizing peptide. The resultant affinity purified antibody was then cross adsorbed against the non-phosphorylated form of the immunizing peptide. Reactivity with non-phosphorylated human Smad2 is minimal by ELISA and dot blot. A BLAST analysis was used to suggest cross-reactivity with Smad2 protein based on 100% homology with the immunizing sequence to human, mouse, rat, orangutan, zebrafish, and bovine. Reactivity against homologues from other sources is not known.
Relevant Links:	• UniProtKB - Q15796 • NCBI - NP_001003652.1 • GenelD - 4087

Application Details

Tested Applications:	Dot Blot, ELISA, IF, IHC
Application Note:	Anti-SMAD2 pS467 affinity purified antibody has been tested for use in ELISA, DB, IHC, and IF. Expect a band approximately ~49, 52.3 kDa in size corresponding to human Smad2 protein by western blotting in the appropriate cell lysate or extract. Specific conditions for reactivity should be optimized by the end user. Positive controls used: HeLa cells in Immunofluorescence and Human brain cortex in Immunohistochemistry.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1.0 µg/ml
IF:	15 µg/mL
IHC:	1:2000
WB:	User Optimized

Formulation

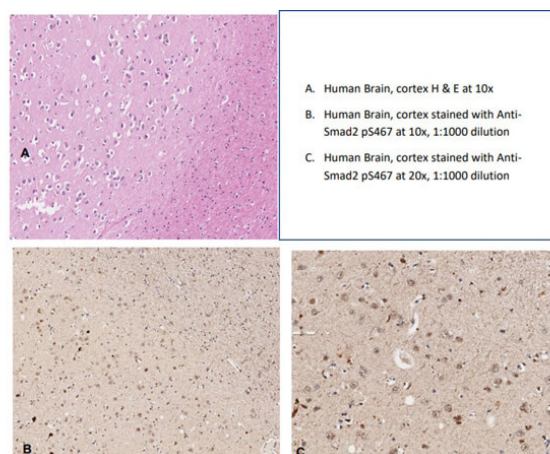
Physical State:	Liquid (sterile filtered)
------------------------	---------------------------

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

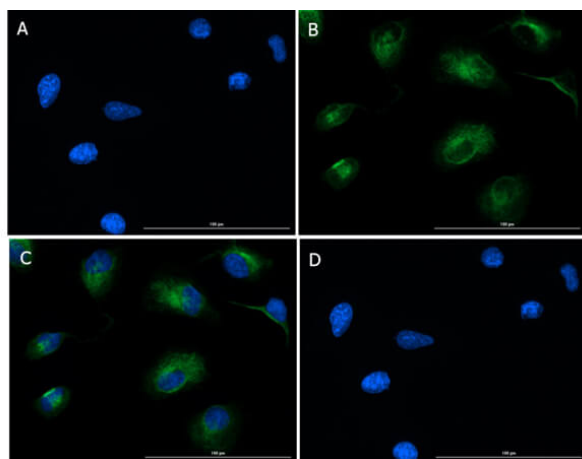
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



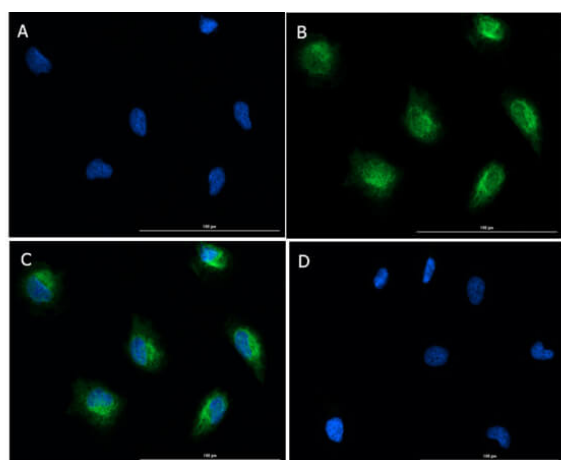
Immunohistochemistry

Immunohistochemistry of Rabbit Anti-SMAD2 pS467 Antibody. Tissue: Human Brain, cortex tissue. Fixative: none. Antigen Retrieval: HIER using Citrate Buffer for 20 mins. Primary Antibody: Anti-SMAD2 pS467 at 1:1000 for 30mins at RT. Secondary Antibody: Goat Anti-Rabbit Poly IgG HRP Ready to Use for 8mins at RT. Counterstain: Hematoxylin. Substrate: DAB. Results: shows diffuse intense intracytoplasmic and intranuclear staining of neurons and glial cells both in white matter and grey matter region at both dilutions and endothelial cells in blood vessels.



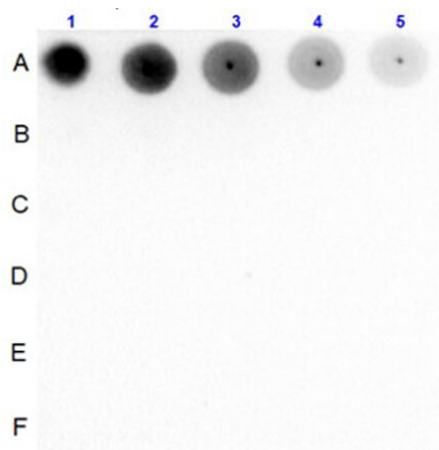
Immunofluorescence Microscopy

Immunofluorescence of Rabbit Anti-SMAD2pS467 Antibody.
 Cells: HeLa Cells. Fixative: 4% PFA. Permeabilization: 0.3% Triton X-100. Primary Antibody: Anti-SMAD2pS467 at 15μg/mL overnight at 2-8°C. Counterstain: Donkey Anti-Rabbit IgG DyLight™488 (p/n 611-741-127) at 5μg/mL for 1hr at RT. Nuclear counterstain: DAPI. Staining: (A) DAPI. (B) Anti-SMAD2pS467 + DyLight™488. (C) Merge A+B. (D) secondary only. Expected localization: Staining of the nucleoli, nucleus and cytosol. Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta stimulation, migrates to the nucleus when complexed with SMAD4. On dephosphorylation by phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1.



Immunofluorescence Microscopy

Immunofluorescence of Rabbit Anti-SMAD2pS467 Antibody.
 Cells: HeLa Cells. Fixative: 4% PFA. Permeabilization: 0.3% Triton X-100. Primary Antibody: Anti-SMAD2pS467 at 15μg/mL overnight at 2-8°C. Counterstain: Donkey Anti-Rabbit IgG DyLight™488 (p/n 611-741-127) at 5μg/mL for 1hr at RT. Nuclear counterstain: DAPI. Staining: (A) DAPI. (B) Anti-SMAD2pS467 + DyLight™488. (C) Merge A+B. (D) secondary only. Expected localization: Staining of the nucleoli, nucleus and cytosol. Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta stimulation, migrates to the nucleus when complexed with SMAD4. On dephosphorylation by phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1.



Dot Blot

Dot Blot of Rabbit Anti-SMAD2pS467 Antibody. Dilution Columns: 1-100ng, 2-33.33ng, 3-11.11ng, 4-3.70ng, 5-1.23ng. Peptide Rows: (A) Smad2 pS467 Peptide conjugated to BSA. (B) Smad2 non phospho S467 Peptide conjugated to BSA. (C) AKT1 pS473 Peptide conjugated to BSA. (D) AKT1 non phospho S473 Peptide conjugated to BSA. (E) Scramble Sprouty Peptide conjugated to BSA. (F) BSA only. Primary Antibody: Anti-SMAD2pS467 at 1μg/mL overnight at 2-8°C. Secondary Antibody: Goat Anti-Rabbit IgG HRP (p/n 611-103-122) at 1:70,000 for 1hr at RT. Blocking buffer: Fluorescent Buffer (p/n MB-070). Exposure: 10sec.

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.