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Datasheet for 600-401-MR3

# SMAD2 phospho S467 Antibody

## Overview

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| **Description:** | Anti-SMAD2 pS467 (RABBIT) Antibody - 600-401-MR3 |
| **Item No.:** | 600-401-MR3 |
| **Size:** | 100 µg |
| **Applications:** | Dot Blot, ELISA, IF, IHC |
| **Reactivity:** | Human |
| **Host Species:** | Rabbit |

## Product Details

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| **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

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| **Gene Name:** | SMAD2 |

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| **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 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<br>• NCBI - NP_001003652.1<br>• GeneID - 4087 |

## Application Details

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| **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

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| **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

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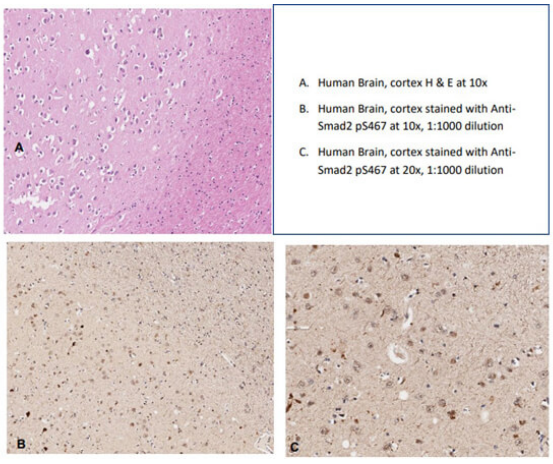
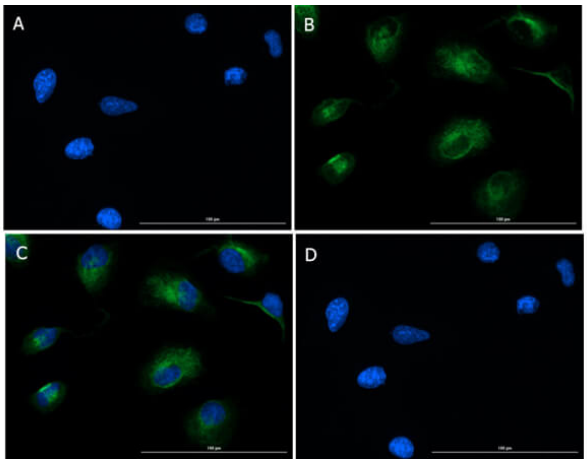
| **Shipping Condition:** | Dry Ice |
| **Storage Condition:** | Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product 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. |

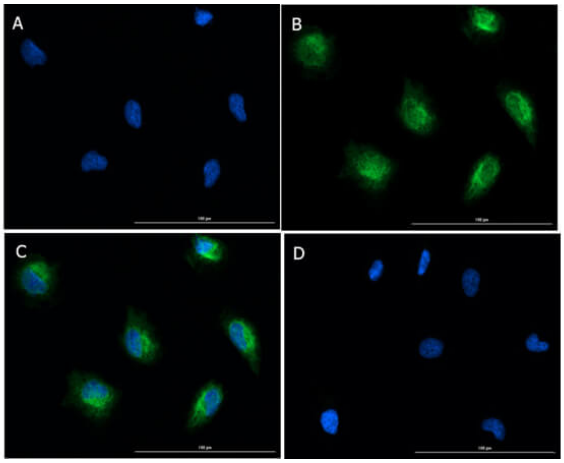
## Images




**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.

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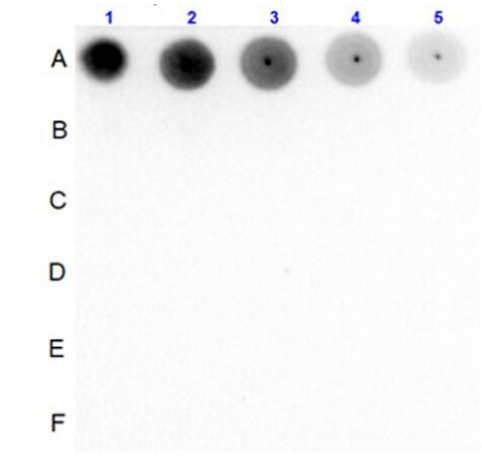


**Dot Blot**
Dot Blot of Rabbit Anti-SMAD2pS467 Antibody. Dilution Columns: 1-100ng, 2-33.33ng, 3-11.11ng, 4-3.70ng, 5-1.23ng. Peptide Rows: (A) Smad2 pS467 Peptide conjugated to BSA. (B) Smad2 non phospho S467 Peptide conjugated to BSA. (C) AKT1 pS473 Peptide conjugated to BSA. (D) AKT1 non phospho 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.

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