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Datasheet for 600-401-E70

# SMAD3 Antibody

## Overview

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| **Description:** | Anti-SMAD3 (RABBIT) Antibody - 600-401-E70 |
| **Item No.:** | 600-401-E70 |
| **Size:** | 100 µg |
| **Applications:** | ELISA, WB |
| **Reactivity:** | Human |
| **Host Species:** | Rabbit |

## Product Details

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| **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. Smad3 (also known as Mothers against decapentaplegic homolog 3, Mothers against DPP homolog 3, Mad3, hMAD-3, JV15-2 or hSMAD3) is a transcriptional modulator activated by TGF-beta (transforming growth factor) and activin type 1 receptor kinase. These activators exert diverse effects on a wide array of cellular processes. The Smad proteins mediate much of the signaling responses induced by the TGF-beta superfamily. Activated type I receptor phosphorylates receptor-activated Smads (R-Smads) at their c-terminal two extreme serines in the S-S-X-S motif, e.g. Smad2 and Smad3 proteins in the TGF-b pathway, or Smad1, Smad5 or Smad8 in the bone morphogenic protein or BMP pathway. Upon phosphorylation R-Smads are translocated into nucleus, where they regulate transcription of target genes. Based on microarray and animal model experiments, Smad3 accounts for at least 80% of all TGF-b-mediated response. |
| **Synonyms:** | rabbit anti-SMAD3 antibody, SMAD-3, SMAD 3, mothers against decapentaplegic homolog 3 antibody, MAD homolog 3, Mothers against DPP homolog 3, SMAD family member 3, MADH3, MADH 3, JV15-2 |
| **Host Species:** | Rabbit |
| **Clonality:** | Polyclonal |
| **Format:** | IgG |

## Target Details

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| **Gene Name:** | SMAD3 |

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| **Reactivity:** | Human |
| **Immunogen Type:** | Conjugated Peptide |
| **Immunogen:** | SMAD3 Antibody was prepared by repeated immunizations with a synthetic peptide corresponding to an internal region of human Smad3 protein surrounding amino acid residue 179. |
| **Purity/Specificity:** | SMAD3 Antibody is directed against human Smad3 protein. The product was affinity purified from monospecific antiserum by immunoaffinity purification. Reactivity occurs against human Smad3 protein corresponding to an internal region surrounding amino acid residue 179. A BLAST analysis was used to suggest cross reactivity with Smad3 from human, mouse, rat, pig, dog, and marmoset based on 100% sequence homology with the immunogen. Reactivity against homologues from other sources is not known. |
| **Relevant Links:** | • GeneID - 4088<br>• NCBI - NP_005893<br>• UniProtKB - P84022 |

## Application Details

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| **Tested Applications:** | ELISA, WB |
| **Application Note:** | SMAD3 Antibody has been tested for use in ELISA and by western blot. This antibody is suitable in immunohistochemistry. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 48.1 kDa in size corresponding to human Smad3 protein by western blotting in the appropriate tissue or cell lysate or extract. |
| **Assay Dilutions:** | All assays should be optimized by the user. Recommended dilutions (if any) may be listed below. |
| **ELISA:** | 1:15,000-1:25,000 |
| **WB:** | 1:1,000 |

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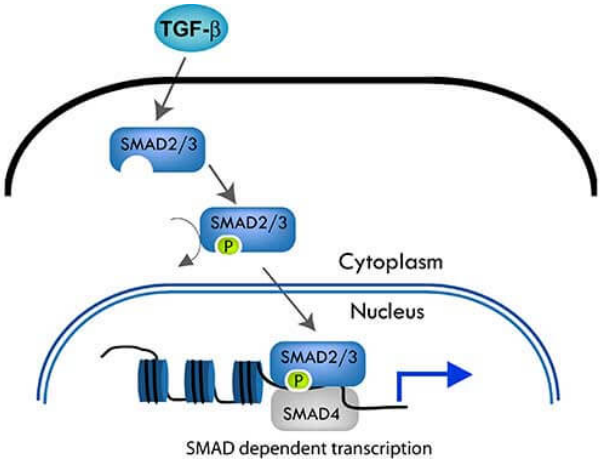
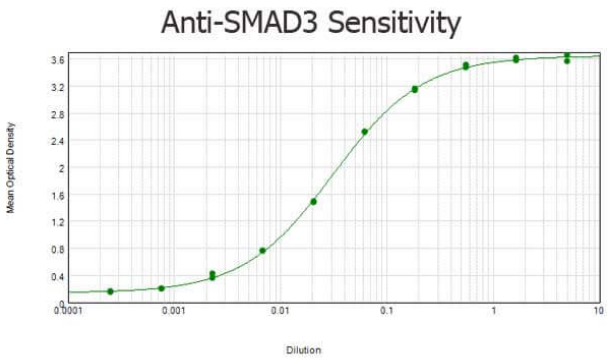
| **Shipping Condition:** | Dry Ice |
| **Storage Condition:** | Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product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

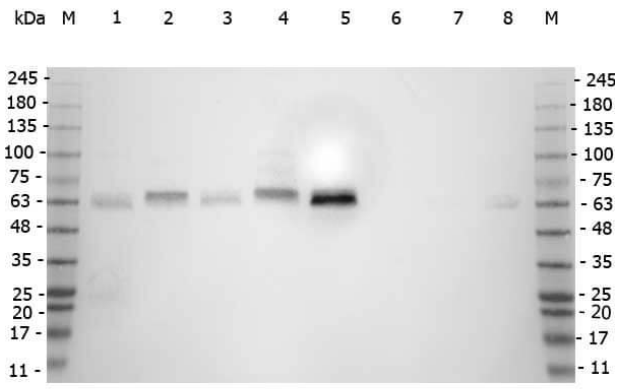


SMAD dependent transcription

**Pathway**
Rabbit anti-SMAD antibody follows the canonical TGF-β signaling pathway. TGF-β dimers bind to a receptor thereby activating the pathway. The type I receptor then recruits and phosphorylates a receptor regulated SMAD (R-SMAD) .i.e. SMAD2 or SMAD3. The R- SMAD then binds to the common SMAD (coSMAD) i.e. SMAD4, and forms a heterodimeric complex. This complex then enters the cell nucleus and acts as a transcription factor.

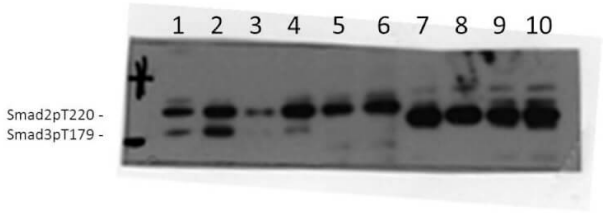



**ELISA**
ELISA results of purified Rabbit anti-SMAD3 Antibody tested against BSA-conjugated peptide of immunizing peptide. Each well was coated in duplicate with 0.1µg of conjugate. The starting dilution of antibody was 5µg/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using 3% fish gel, Goat anti-Rabbit IgG Antibody Peroxidase Conjugated (Min X Bv Ch Gt GP Ham Hs Hu Ms Rt & Sh Serum Proteins) (p/n 611-103-122) and TMB ELISA Peroxidase Substrate (p/n TMBE-1000).




**Western Blot**

Western Blot of Rabbit anti-SMAD3 antibody. Marker: Opal Pre-stained ladder (p/n MB-210-0500). Lane 1: HEK293 lysate (p/n W09-000-365). Lane 2: HeLa Lysate (p/n W09-000-364). Lane 3: MCF-7 Lysate (p/n W09-000-360). Lane 4: Jurkat Lysate (p/n W09-000-370). Lane 5: A549 Lysate (p/n W09-001-372). Lane 6: HL-60 Lysate (p/n W09-001-GL3). Lane 7: Raji Lysate (p/n W09-001-368). Lane 8: NIH/3T3 Lysate (p/n W10-000-358). Load: 35 µg per lane. Primary antibody: SMAD3 antibody at 1:5,000 for overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody (p/n 611-103-122) at 1:30,000 for 60 min at RT. Blocking Buffer: 1% Casein-TTBS (p/n MB-082) for 30 min at RT. Predicted/Observed size: 48 kDa for SMAD3.




**Western Blot**

Western Blot of Rabbit Anti-SMAD3 antibody. Lane 1: AML12 unstimulated. Lane 2: AML12 stimulated with TGFB. Lane 3: MEFwt unstimulated. Lane 4: MEFwt stimulated with TGFB. Lane 5: MEF Smad3 KO unstimulated. Lane 6: MEF Smad3 KO stimulated with TGFB. Lane 7: HEK293 Smad3T179A mutant unstimulated. Lane 8: HEK293 Smad3T179A mutant stimulated with TGFB. Lane 9: HEK293 Smad3T179V mutant unstimulated. Lane 10: HEK293 Smad3T179V mutant stimulated with TGFB. Load: 35 µg per lane. Primary antibody: SMAD 3 antibody at 1:1000 for overnight at 4°C. Secondary antibody: IRDye800™ rabbit secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: 48.1kDa. Other band(s): Smad2pT220.

## Disclaimer