Anti-Human SMAD4, monoclonal (clone R516.2.1D12)

Recommended name: Mothers against decapentaplegic homolog; Short name: MAD homolog ; Mothers against DPP homolog
Alternative name(s): SMAD family member

Cat. No.	**m14-150**	**Quantity:**	**100 µg**
Lot. No.	**20150610.L.I**	**Storage:**	**-20˚C**

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Uniprot / NCBI Summary

UniProt
Primary Accession # Q13485
Secondary Accession # A8K405

NCBI
GI # 4885457
GeneID 4089
Accession # NP_005350.1
GenBank Nucleotide # NM_005359.5

Molecular Weight 60,439 Da (552 aa)

Smad proteins are phosphorylated and activated by transmembrane serine-threonine receptor kinases in response to TGF-beta signaling. The product of this gene forms homomeric complexes and heteromeric complexes with other activated Smad proteins, which then accumulate in the nucleus and regulate the transcription of target genes. This protein binds to DNA and recognizes an 8-bp palindromic sequence (GTCTAGAC) called the Smad-binding element (SBE). The Smad proteins are subject to complex regulation by post-translational modifications. Mutations or deletions in this gene have been shown to result in pancreatic cancer, ju-

Continued on page 2.

Physical Characteristics

Quantity: 100 µg

Concentration: 1.0 mg/ml

Host / Isotype: mouse IgG2a

Clonality: monoclonal; ID R516.2.1D12

Immunogen: recombinant protein corresponding to aa residues 314-552 of human SMAD4

Purification: affinity-chromatography using Protein G

Formulation: 30% glycerol, 1x PBS, 0.02% sodium azide

Specificity: monospecific for human SMAD4; see microarray analysis below

Reactivity: human

Stability/Storage: 12 months long term: -20˚C; short term: 4˚C; avoid freeze-thaw cycles; aliquot as required

Handling Notes: small volumes of antibody may occasionally become entrapped in the seal of the product vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the container cap.

Tested Research Applications

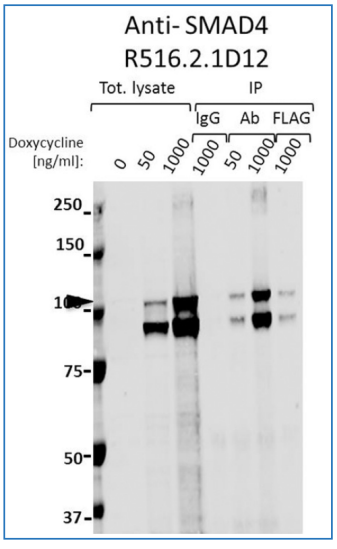
Western Immunoblotting: tested on cells transfected with a construct encoding SMAD4; utility on native cells under evaluation

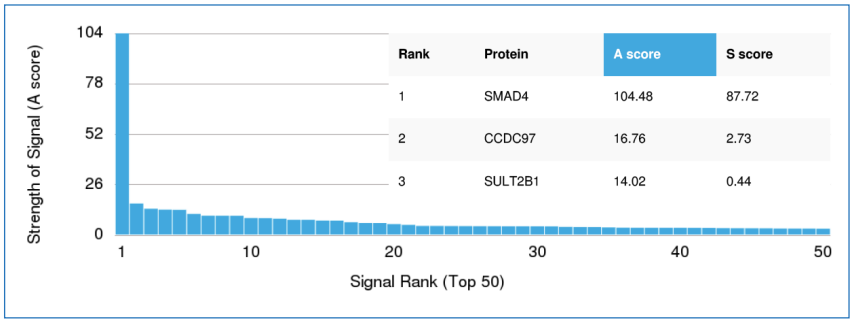
Immunoprecipitation: recommended; see below

ChIP-Seq: recommended; see page 2

Quality Assurance

IP Analysis:
Tet-ON HeLa cells were transfected with construct encoding SMAD4 with an N-terminal fusion of FLAG, YFP (Venus) and V5 tags under a tet-inducible promoter. These cells were stimulated with 0, 50 or 1000 ng/ml doxycycline. Immunoprecipitation (IP) was carried out using 5µg of either IgG, CDI mAb Anti-SMAD4 (cloneID# R516.2.1D12) or 1 µg of FLAG-M2. Immunoblotting was performed using rabbit Anti-FLAG (1:1000, Cell Signaling #2368).





Specificity Analysis with HuProt™ Human Proteome Microarray: Anti Human SMAD4 (cloneR516.2.1D12) was analyzed using the CDI HuProt™ Human Proteome Microarray.

For more information on A/S scores and how they relate to specificity, see page 2.

Guanajibo Research and Innovation Park
4005 St B Road 114 Km 1.3
Mayaguez, PR 00682

T 787.806-4100 Ext 233
F 787.806-4006
www.cdi-lab.com

Lot-specific Datasheet version tracker: v2.0.0

Anti-Human SMAD4, monoclonal (cloneR516.2.1D12)

Recommended name: Mothers against decapentaplegic homolog; Short name: MAD homolog ; Mothers against DPP homolog
Alternative name(s):SMAD family member

Cat. No.	**m14-150**	**Quantity:**	**100 µg**
Lot. No.	**20150610.L.I**	**Storage:**	**-20˚C**

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Uniprot / NCBI Summary

Continued from page 1.

venile polyposis syndrome, and hereditary hemorrhagic telangiectasia syndrome.

Subcellular location: Nucleus; cytoplasm.

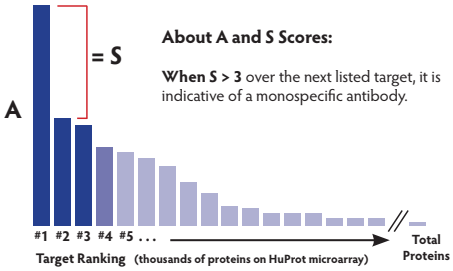
Sequence similarities: Belongs to the dwarfin/SMAD family. Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain.

General Reference:

Zhang Y, Feng X-H, Wu R-Y, Derynck R (1995) Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. *Nature* **383**:168-172. [PubMed]

Qin B, Lam SS, Lin K (1998) Crystal structure of a transcriptionally active Smad4 fragment. *Structure* **7**:1493-1503. [PubMed]

Le Goff C, Mahaut C, Abhyankar A, Le Goff W, Serre V, Afenjar A, Destree A, di Rocco M, Heron D, Jacquemont S, Marlin S, Simon M, Tolmie J, Verloes A, Casanova JL, Munnich A, Cormier-Daire V (2011) Mutations at a single codon in Mad homology 2 domain of SMAD4 cause Myhre syndrome. *Nat Genet* **44**:85-88. [PubMed]



About A and S Scores:

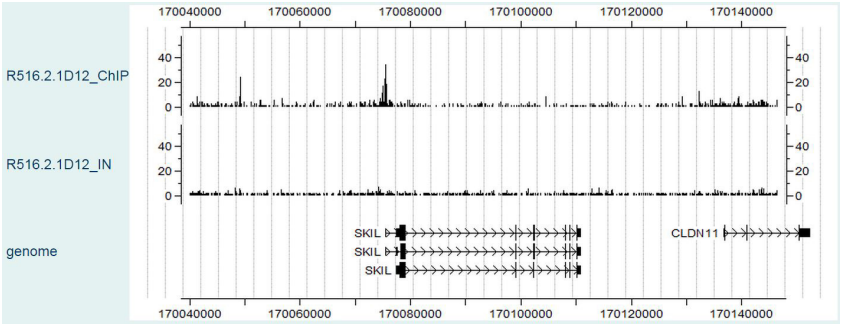
When S > 3 over the next listed target, it is indicative of a monospecific antibody.

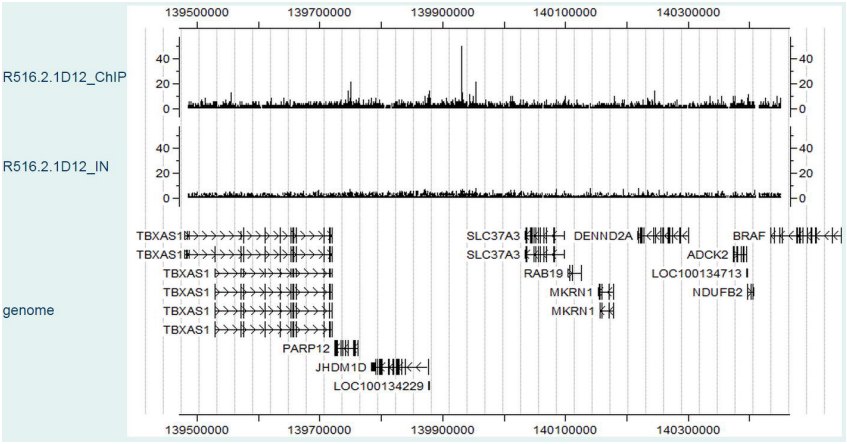
Statistical Analysis: Thousands of GenePix data points (from the microarray) are analyzed in terms of signal strength and ranked accordingly.

SUMMARY: The A-score indicates the number of standard deviations above background seen for the mean signal bound by the target antigen The S-score represents the difference between the A-score of the target antigen and the next best hit on the array. S-scores **greater than 3 standard deviations over the next listed target** are deemed statistically significant and indicate **highly specific antibodies.** More info at cdi-lab.com/HighSpec.html

Tested Research Applications

ChIP-Seq: Recommended





The ChIP was performed with chromatin from 10 million HepG2 cells and 3 µg of Anti-SMAD4 (cloneID #R516.2.1D12) antibody. The ChIP DNA was sequenced on an Illumina HiSeq platform and read counts were calculated at consecutive 100 bp bins across the human genome hg19. Normalized read-count levels for ChIP-seq of SMAD4 (R516.2.1D12_ChIP) and control (R516.2.1D12_IN) around the SKIL and JHDM1D loci are displayed in the CisGenome browser.

The development of this antibody was supported by the National Institutes of Health Protein Capture Reagent Program under award U54HG06434 to CDI Laboratories and Johns Hopkins University.

Guanajibo Research and Innovation Park
4005 St B Road 114 Km 1.3
Mayaguez, PR 00682

T 787.806-4100 Ext 233
F 787.806-4006
www.cdi-lab.com