abcam

Product datasheet

Anti-Smad1 (phospho S463 + S463) antibody [EPR20662-20] ab226821

Recombinant RabMAb

4 References 5 Images

Overview

Product name	Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-20]
Description	Rabbit monoclonal [EPR20662-20] to Smad1 (phospho S463 + S465)
Host species	Rabbit
Specificity	Based on sequence homology this antibody also reacts with Smad5 (phospho S463/S465) and Smad9 (phospho S465/S467).
Tested applications	**Suitable for:** WB, Dot blot, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa grown in serum-free media overnight, treated with 100 ng/ml Calyculin A (**ab141784**) for 15min followed by Calyculin A removal and treatment with 100 ng/ml BMP2 for 30min, whole cell lysate; NIH/3T3 cultured in serum-free media overnight then treated with 50 ng/ml BMP2 for 30min whole cell lysate. ICC/IF: NIH3T3 cells FBS-deprived overnight before treatment with 50 ng/ml hBMP2 for 30min. IP: NIH/3T3 grown in serum-free media overnight then treated with 50 ng/ml BMP2 for 30min whole cell lysate.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2

	Preservative: 0.01% Sodium azide Constituents: PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20662-20
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab226821 in the following tested applications.

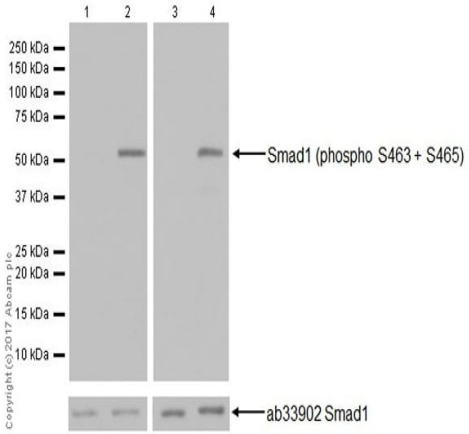
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 60 kDa (predicted molecular weight: 52 kDa).
Dot blot		1/1000.
ICC/IF		1/100.
IP		1/30.

Target

Function	Transcriptional modulator activated by BMP (bone morphogenetic proteins) type 1 receptor kinase. SMAD1 is a receptor-regulated SMAD (R-SMAD). SMAD1/OAZ1/PSMB4 complex mediates the degradation of the CREBBP/EP300 repressor SNIP1.
Tissue specificity	Ubiquitous. Highest expression seen in the heart and skeletal muscle.
Sequence similarities	Belongs to the dwarfin/SMAD family. Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain.
Post-translational modifications	Phosphorylated on serine by BMP type 1 receptor kinase. Ubiquitin-mediated proteolysis by SMAD-specific E3 ubiquitin ligase SMURF1.
Cellular localization	Cytoplasm. Nucleus. Cytoplasmic in the absence of ligand. Migrates to the nucleus when complexed with SMAD4. Co-localizes with LEMD3 at the nucleus inner membrane.

Images



Western blot - Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-20] (ab226821)

All lanes : Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-20] (ab226821) at 1/1000 dilution

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) grown in serum-free media overnight, whole cell lysate
Lane 2 : HeLa grown in serum-free media overnight, then treated with 100 ng/ml Calyculin A (**ab141784**) for 15 minutes, followed by Calyculin A removal and treatment with 100 ng/ml BMP2 for 30 minutes, whole cell lysate
Lane 3 : NIH/3T3 (mouse embryo fibroblast cell line) grown in serum-free media overnight, whole cell lysate
Lane 4 : NIH/3T3 cultured in serum-free media overnight, then treated with 50 ng/ml BMP2 for 30 minutes, whole cell lysate

Lysates/proteins at 20 µg per lane.

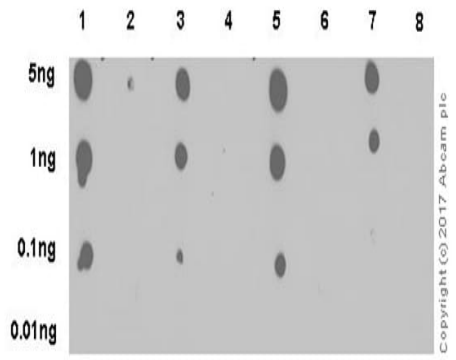
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 52 kDa
Observed band size: 60 kDa

Exposure time:
Lanes 1 and 2: 3 minutes.
Lanes 3 and 4: 30 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.



Dot Blot - Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-20] (ab226821)

Dot blot analysis of Smad1 (phospho S463 + S465) labeled with ab226821 at 1/1000 dilution.

Lane 1: Smad1 (phospho S463/S465) peptide;

Lane 2: Smad1 (phospho S463) peptide;

Lane 3: Smad1 (phospho S465) peptide;

Lane 4: Smad1 peptide (not phosphorylated);

Lane 5: Smad5 (phospho S463/S465) peptide;

Lane 6: Smad5 (phospho S463) peptide;

Lane 7: Smad5 (phospho S465) peptide;

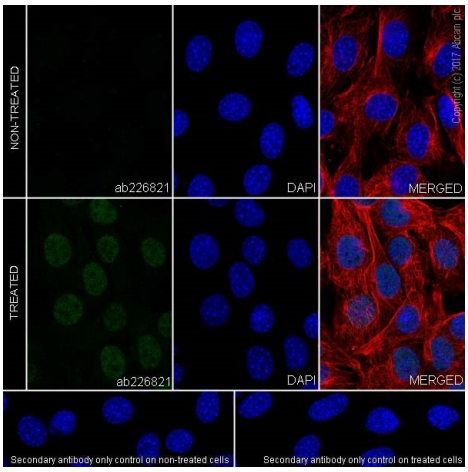
Lane 9: Smad5 peptide (not phosphorylated).

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution was used as secondary antibody.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

Based on sequence homology, this antibody cross-reacts with Smad5 (phospho S463/S465) and Smad9 (phospho S465/S467).

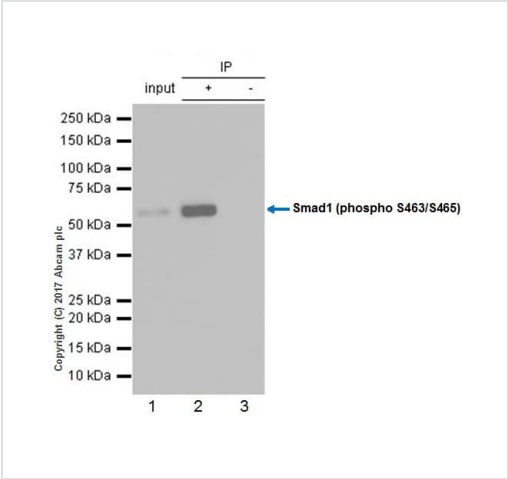


Immunocytochemistry/ Immunofluorescence - Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-20] (ab226821)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryo fibroblast cell line) cells labeling Smad1 (phospho S463 + S465) with ab226821 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Nuclear staining in hBMP2-treated NIH/3T3 cells. Cells were FBS-deprived overnight before treatment with 50 ng/ml hBMP2 for 30 minutes.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Immunoprecipitation - Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-20] (ab226821)

Smad 1 (phospho S463 + S465) was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryo fibroblast cell line) grown in serum-free media overnight, then treated with 50 ng/ml BMP2 for 30 minutes, whole cell lysate with ab226821 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab226821 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

Lane 1: NIH/3T3 grown in serum-free media overnight, then treated with 50 ng/ml BMP2 for 30 minutes, whole cell lysate 10 µg (Input).

Lane 2: ab226821 IP in NIH/3T3 grown in serum-free media overnight, then treated with 50 ng/ml BMP2 for 30 minutes, whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab226821 in NIH/3T3 grown in serum-free media overnight, then treated with 50 ng/ml BMP2 for 30 minutes, whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.

Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-20] (ab226821)

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