abcam

Product datasheet

Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-29] ab214423

Recombinant RabMAb

3 References 4 Images

Overview

Product name	Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-29]
Description	Rabbit monoclonal [EPR20662-29] 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, IP
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	Dot Blot: Smad1 (phospho S463/S465) peptide, Smad1 (phospho S465) peptide, Smad5 (phospho S463/S465) peptide, Smad5 (phospho S465) peptide. WB: Calyculin (**ab141784**) treated HeLa cell lysate; BMP2 treated NIH/3T3 cell lysate. IP: BMP2 treated NIH/3T3 cell lysate.
General notes	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, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20662-29
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab214423 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.
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.

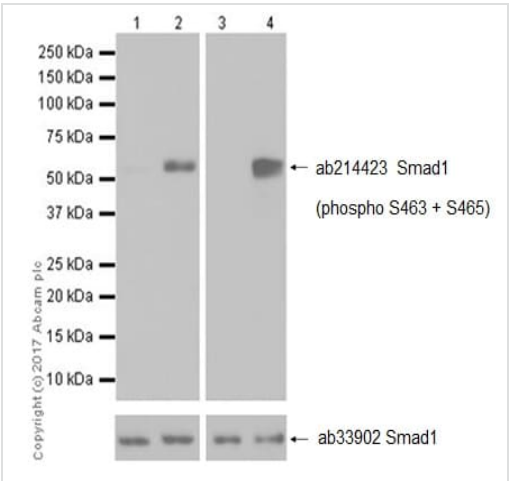
Tissue specificity Ubiquitous. Highest expression seen in the heart and skeletal muscle.

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 [EPR20662-29] (ab214423)

All lanes : Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-29] (ab214423) at 1/1000 dilution

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

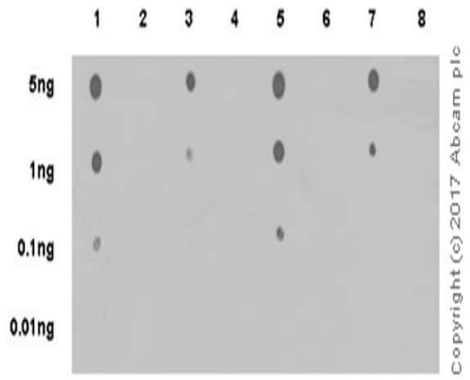
Developed using the ECL technique.

Performed under reducing conditions.

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

Exposure time: 10 seconds

Blocking/Dilution: 5% NFDM/TBST.

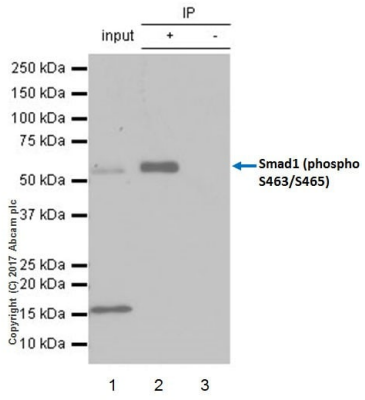


Dot Blot - Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-29] (ab214423)

Dot blot analysis of Smad1 (phospho S463/S465) peptide (Lane 1), Smad1 (phospho S463) peptide (Lane 2), Smad1 (phospho S465) peptide (Lane 3), Smad1 non-phospho peptide (Lane 4), Smad5 (phospho S463/S465) peptide (Lane 5), Smad5 (phospho S463) peptide (Lane 6), Smad5 (phospho S465) peptide (Lane 7) and Smad5 non-phospho peptide (Lane 8) using ab214423 at 1/1,000 dilution followed by Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100,000 dilution.

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

Exposure time: 30 seconds.



Immunoprecipitation - Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-29] (ab214423)

Smad1 (phospho S463/S465) was immunoprecipitated from 0.35 mg of NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate with ab214423 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab214423 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1,000 dilution

Lane 1: NIH/3T3 (mouse embryonic fibroblast) grown in serum-free media overnight, then treated with 50 ng/ml BMP2 for 30 minutes, whole cell lysate 10 µg (Input).
Lane 2: ab214423 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 ab214423 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: 5% NFDM/TBST.

Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-29] (ab214423)

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