abcam

Product datasheet

Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-20] - BSA and Azide free ab228457



RabMAb

4 图像

概述

产品名称 Anti-Smad1 (phospho S463 + S465)抗体[EPR20662-20] - BSA and Azide free

宿主 Rabbit

特异性 Based on sequence homology this antibody also reacts with Smad5 (phospho S463/S465) and

Smad9 (phospho S465/S467).

经测试应用 适用于: IP, WB, ICC/IF, Dot blot

种属反应性 与反应: Mouse, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

阳性对照 ICC/IF: NIH3T3 cells FBS-deprived overnight before treatment with 50 ng/ml hBMP2 for 30min.

常规说明 ab228457 is the carrier-free version of ab226821.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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

性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C. Do Not Freeze.

存储溶液 pH: 7.2

Constituent: PBS

纯**度** Protein A purified

克隆 单克隆

克隆编号 EPR20662-20

同种型 lgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab228457于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 60 kDa (predicted molecular weight: 52 kDa).
ICC/IF		Use at an assay dependent concentration.
Dot blot		Use at an assay dependent concentration.

靶标

功能 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.

组织特异性 Ubiquitous. Highest expression seen in the heart and skeletal muscle.

序列相似性 Belongs to the dwarfin/SMAD family.

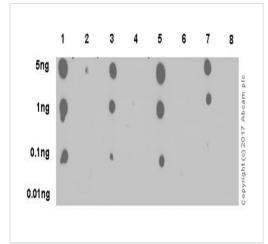
Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain.

翻译后修饰 Phosphorylated on serine by BMP type 1 receptor kinase.

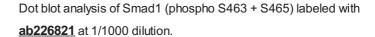
Ubiquitin-mediated proteolysis by SMAD-specific E3 ubiquitin ligase SMURF1.

细胞定位 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.



Dot Blot - Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-20] - BSA and Azide free (ab228457)



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).

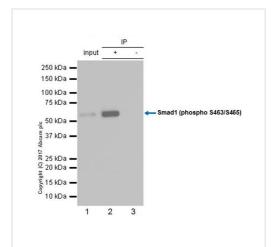
Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution was used as secondary antibody.

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab226821).



Immunoprecipitation - Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-20] - BSA and Azide free (ab228457)

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 μ g (Input).

Lane 2: <u>ab226821</u> 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 (<u>ab172730</u>) instead of <u>ab226821</u> 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab226821).

ab226821

DAPI

MERGED

Ab226821

DAPI

MERGED

MERGED

Secondary antibody only control on non-treated cells

Secondary antibody only control on non-treated cells

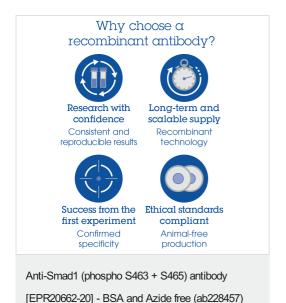
Immunocytochemistry/ Immunofluorescence - Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-20] - BSA and Azide free (ab228457)

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 lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab226821).



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