# abcam

### Product datasheet

## Anti-Smad2 (phospho S255) antibody [EPR2856(N)] ab188334



重组 RabMAb

★★★★★ 2 Abreviews 53 References 9 图像

概述

产品名称 Anti-Smad2 (phospho S255)抗体[EPR2856(N)]

描述 兔单克隆抗体[EPR2856(N)] to Smad2 (phospho S255)

宿主 Rabbit

经测试应用 适用于: Dot blot, IHC-P, IP, WB, ChIC/CUT&RUN-seq

种属反应性 与反应: Mouse, Rat, Human

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

阳性对照 Hela treated with Okadaic acid and Calyculin A, Hela treated with Okadaic acid and Calyculin A,

Human endometrium, Human transitional cell carcinoma of bladder. RAW 264.7 (Mouse Abelson

murine leukemia virus-induced tumor macrophage) and PC-12 (Rat adrenal gland

pheochromocytoma) whole cell lysates. ChlC/CUT&RUN-Seq: HaCaT cells.

常规说明 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**.

性能

形式 Liquid

存放说明 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.

存储溶液 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

纯度 Protein A purified

克隆 单克隆

克隆编号 EPR2856(N)

同种型 lgG

#### 应用

#### Abpromise™承诺保证使用ab188334于以下的经测试应用 The Abpromise guarantee

"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
Dot blot		Use at an assay dependent concentration.
IHC-P	<b>★★★★★ (1)</b>	1/50 - 1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/50 - 1/70.
WB		1/1000 - 1/10000. Detects a band of approximately 58 kDa (predicted molecular weight: 52 kDa).
ChlC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg

#### 靶标

功能 Receptor-regulated SMAD (R-SMAD) that is an intracellular signal transducer and transcriptional

> modulator activated by TGF-beta (transforming growth factor) and activin type 1 receptor kinases. Binds the TRE element in the promoter region of many genes that are regulated by TGF-beta and, on formation of the SMAD2/SMAD4 complex, activates transcription. May act as a tumor

suppressor in colorectal carcinoma.

组织特异性 Expressed at high levels in skeletal muscle, heart and placenta.

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 one or several of Thr-220, Ser-245, Ser-250, and Ser-255. In response to

> TGF-beta, phosphorylated on Ser-465/467 by TGF-beta and activin type 1 receptor kinases. Able to interact with SMURF2 when phosphorylated on Ser-465/467, recruiting other proteins, such as SNON, for degradation. In response to decorin, the naturally occurring inhibitor of TGF-beta signaling, phosphorylated on Ser-240 by CaMK2. Phosphorylated by MAPK3 upon EGF stimulation; which increases transcriptional activity and stability, and is blocked by calmodulin.

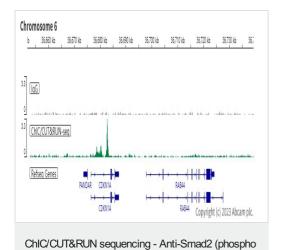
In response to TGF-beta, ubiquitinated by NEDD4L; which promotes its degradation. Acetylated on Lys-19 by coactivators in response to TGF-beta signaling, which increases transcriptional activity. Isoform short: Acetylation increases DNA binding activity in vitro and enhances its association with target promoters in vivo. Acetylation in the nucleus by EP300 is

enhanced by TGF-beta.

细胞定位 Cytoplasm. Nucleus. Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta

> stimulation, migrates to the nucleus when complexed with SMAD4. On dephosphorylation by phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the

nucleus by interaction with RANBP1.

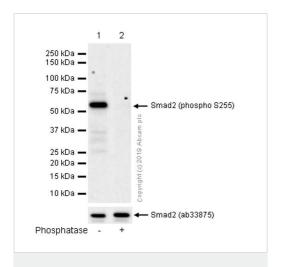


S255) antibody [EPR2856(N)] (ab188334)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL,  $2 \times 10^5$  HaCaT (human skin keratinocyte) cells and  $5 \mu g$  of ab188334 [EPR2856(N)]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control <u>ab172730</u> is also shown.

Additional screenshots of mapped reads can be downloaded **here**.

The University of Geneva owns patents relevant to ChlC (Chromatin Immuno-Cleavage) methods.



Western blot - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334)

**All lanes :** Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334) at 1/10000 dilution

**Lane 1 :** PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate

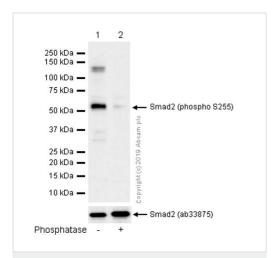
**Lane 2 :** PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate, Then the membrane was incubated with phosphatase

Lysates/proteins at 15 µg per lane.

#### **Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 52 kDa



Western blot - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334)



**Lane 1 :** RAW 264.7 (Mouse Abelson murine leukemia virusinduced tumor macrophage) whole cell lysate

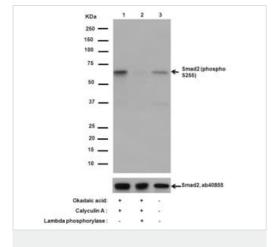
**Lane 2:** RAW 264.7 (Mouse Abelson murine leukemia virusinduced tumor macrophage) whole cell lysate. Then the membrane was incubated with phosphatase.

Lysates/proteins at 15 µg per lane.

### **Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 52 kDa



Western blot - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334)

**All lanes :** Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334) at 1/10000 dilution

Lane 1: Hela treated with Okadaic acid and Calyculin A

**Lane 2 :** Hela treated with Okadaic acid and Calyculin A , then treated with Lambda phosphorylase

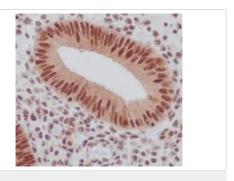
Lane 3: Untreated Hela

Lysates/proteins at 10 µg per lane.

#### **Secondary**

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

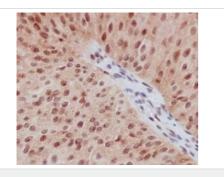
Predicted band size: 52 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334)

Immunohistochemical analysis of formalin fixed paraffin embedded Human endometrium labeling Smad2 (phospho S255) with ab188334 at 1/100 dilution and HRP Polymer for Rabbit lgG. Counterstained with Hematoxylin.

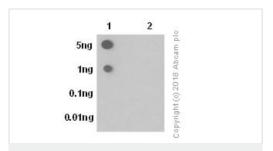
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334)

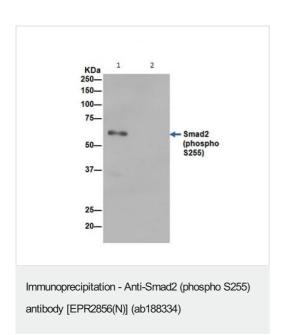
Immunohistochemical analysis of formalin fixed paraffin embedded Human transitional cell carcinoma of bladder labeling Smad2 (phospho S255) with ab188334 at 1/100 dilution and HRP Polymer for Rabbit IgG. Counterstained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

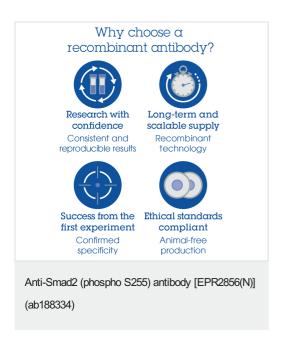


Dot Blot - Anti-Smad2 (phospho S255) antibody [EPR2856(N)] (ab188334)

Dot blot analysis of Smad2 (S255) phospho peptide (Lane 1), Smad2 non-phospho peptide (Lane 2), labelling Smad2 (S255) phospho peptide with ab188334 at a dilution of 1:1000 dilution (1.365ug/ml). A Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (ab97051) was used as the secondary antibody at a dilution of 1:20,000 dilution. Blocking buffer: 5% NFDM/TBST. Dilution buffer: 5% NFDM/TBST.



Immunoprecipitation of Hela cells treated with Okadaic acis and Calyculin A (Lane 1) or PBS (Lane 2) labeling Smad2 (phospho S255) with ab188334 at 1/50 dilution and Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/1500 dilution



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