# abcam

## Product datasheet

## HRP Anti-Smad3 antibody [EP568Y] ab204462



重组 RabMAb

#### 3 图像

#### 概述

产品名称 HRP Anti-Smad3抗体[EP568Y]

描述 HRP兔单克隆抗体[EP568Y] to Smad3

宿主 Rabbit 偶联物 HRP

经测试应用 适用于: WB, IHC-P 种属反应性 与反应: Rat, Human

预测可用于: Mouse

免疫原 Synthetic peptide within Human Smad3 aa 200-300 (internal sequence). The exact sequence is

proprietary.

(Peptide available as ab173094)

阳性对照 WB: Rat liver tissue lysate. IHC-P: FFPE human colon adenocarcinoma tissue sections.

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.

Avoid freeze / thaw cycle. Store In the Dark.

存储溶液 pH: 7.40

Preservative: 0.1% Proclin 300 Solution

Constituents: PBS, 1% BSA, 30% Glycerol (glycerin, glycerine)

纯度 Protein A purified

 克隆
 单克隆

 克隆编号
 EP568Y

**同种型** IgG

#### 应用

#### The Abpromise guarantee Abpromise™承诺保证使用ab204462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
WB		1/5000. Detects a band of approximately 58 kDa (predicted molecular weight: 48 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

#### 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 SMAD3/SMAD4 complex, activates transcription. Also can form a SMAD3/SMAD4/JUN/FOS complex at the AP-1/SMAD site to regulate TGF-beta-mediated transcription. Has an inhibitory effect on wound healing probably by modulating both growth and migration of primary keratinocytes and by altering the TGF-mediated chemotaxis of monocytes. This effect on wound healing appears to be hormone-sensitive. Regulator of chondrogenesis and osteogenesis and inhibits early healing of bone fractures. Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator.

疾病相关 Colorectal cancer

Loeys-Dietz syndrome 3

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.

结**构域** The MH1 domain is required for DNA binding. Also binds zinc ions which are necessary for the

DNA binding.

The MH2 domain is required for both homomeric and heteromeric interactions and for

transcriptional regulation. Sufficient for nuclear import.

The linker region is required for the TGFbeta-mediated transcriptional activity and acts

synergistically with the MH2 domain.

翻译后修饰 Phosphorylated on serine and threonine residues. Enhanced phosphorylation in the linker region

on Thr-179, Ser-204 and Ser-208 on EGF and TGF-beta treatment. Ser-208 is the main site of MAPK-mediated phosphorylation. CDK-mediated phosphorylation occurs in a cell-cycle dependent manner and inhibits both the transcriptional activity and antiproliferative functions of

SMAD3. This phosphorylation is inhibited by flavopiridol. Maximum phosphorylation at the G(1)/S junction. Also phosphorylated on serine residues in the C-terminal SXS motif by TGFBR1 and

2

ACVR1. TGFBR1-mediated phosphorylation at these C-terminal sites is required for interaction with SMAD4, nuclear location and transactivational activity, and appears to be a prerequisite for the TGF-beta mediated phosphorylation in the linker region. Dephosphorylated in the C-terminal SXS motif by PPM1A. This dephosphorylation disrupts the interaction with SMAD4, promotes nuclear export and terminates TGF-beta-mediated signaling. Phosphorylation at Ser-418 by CSNK1G2/CK1 promotes ligand-dependent ubiquitination and subsequent proteasome degradation, thus inhibiting SMAD3-mediated TGF-beta responses. Phosphorylated by PDPK1. Acetylation in the nucleus by EP300 in the MH2 domain regulates positively its transcriptional activity and is enhanced by TGF-beta.

Ubiquitinated. Monoubiquitinated, leading to prevent DNA-binding. Deubiquitination by USP15 alleviates inhibition and promotes activation of TGF-beta target genes.

Poly-ADP-ribosylated by PARP1 and PARP2. ADP-ribosylation negatively regulates SMAD3 transcriptional responses during the course of TGF-beta signaling.

Cytoplasm. Nucleus. Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta stimulation, migrates to the nucleus when complexed with SMAD4 (PubMed:15799969). Through the action of the phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1 (PubMed:16751101, PubMed:19289081). Co-localizes with LEMD3 at the nucleus inner membrane (PubMed:15601644). MAPK-mediated phosphorylation appears to have no effect on nuclear import (PubMed:19218245). PDPK1 prevents its nuclear translocation in response to TGF-beta (PubMed:17327236).

#### 图片

(ab204462)

细胞定位

250 kDa — 1
150 kDa — 1
100 kDa — 75 kDa — 75 kDa — 37 kDa — 37 kDa — 37 kDa — 25 kDa — 20 kDa — 20 kDa — 15 kDa — 15 kDa — 10 kD

HRP Anti-Smad3 antibody [EP568Y] (ab204462) at 1/5000 dilution + Liver (Rat) Tissue Lysate at 10 µg

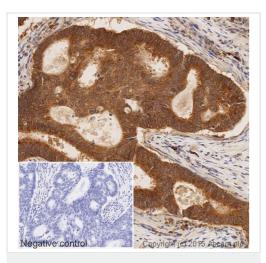
Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 48 kDa **Observed band size:** 58 kDa

Exposure time: 20 minutes

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before being incubated with ab204462 overnight at 4°C. Antibody binding was visualised using ECL development solution **ab133406**.

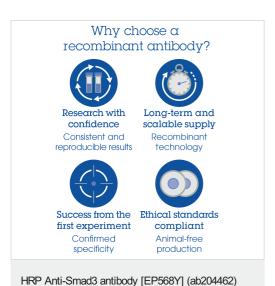


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - HRP Anti-Smad3 antibody
[EP568Y] (ab204462)

IHC image of Smad3 staining in a section of formalin-fixed paraffinembedded human colon adenocarcinoma, performed on a Leica BOND. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab204462, 1/200 dilution, for 15 mins at room temperature. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset negative control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

\*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre.



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