# abcam

### Product datasheet

## Anti-Smad2 + Smad3 antibody [EPR19557-4] - BSA and Azide free ab232326



重组 RabMAb

6 图像

#### 概述

产品名称 Anti-Smad2 + Smad3抗体[EPR19557-4] - BSA and Azide free

描述 兔单克隆抗体[EPR19557-4] to Smad2 + Smad3 - BSA and Azide free

宿主 Rabbit

经测试应用 适用于: Flow Cyt (Intra), WB, ICC/IF, IP, ChIP, ChIC/CUT&RUN-seq

种属反应性 与反应: Mouse, Rat, Human

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

阳性对照 ICC/IF: HeLa cells. ChlC/CUT&RUN seq: HaCaT cell

常规说明 ab232326 is the carrier-free version of ab202445.

> Our carrier-free 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 cellbased 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<sup>®</sup> 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 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. Do Not Freeze.

**存储溶液** pH: 7.2

Constituent: PBS

**无载体** 是

纯**度** Protein A purified

**克隆** 单克隆

**克隆编号** EPR19557-4

**同种型** IgG

#### 应用

## The Abpromise guarantee Abpromise™承诺保证使用ab232326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
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 58-62 kDa (predicted molecular weight: 52 kDa).
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
ChIP		Use at an assay dependent concentration.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.

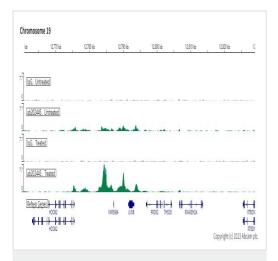
相关性	SMAD is a family of proteins similar to the gene products of the Drosophila gene 'mothers against	
	decapentaplegic' (Mad) and the C. elegans gene Sma. SMAD proteins are signal transducers	
	and transcriptional modulators that mediate multiple signaling pathways. They mediate the signal	
	of the transforming growth factor (TGF)-beta, and thus regulate multiple cellular processes, such	
	as cell proliferation, apoptosis, and differentiation.	

细胞定位 Cytoplasm. Nucleus. Note: Cytoplasmic in the absence of ligand. Migrates to the nucleus when

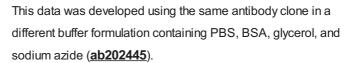
complexed with SMAD4.

#### 图片

靶标

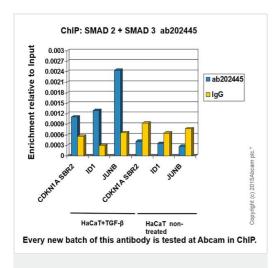


ChIC/CUT&RUN sequencing - Anti-Smad2 + Smad3 antibody [EPR19557-4] - BSA and Azide free (ab232326)



ChIC/CUT&RUN was performed using ta pAG-MNAse at a final concentration of 700 ng/ $\mu$ L, 2.5 x 10^5 HaCaT (Human keratinocyte cell line) cells (treated with 7ng/ml TGF- $\beta$  for 1h) and 5  $\mu$ g of ab202445 [EPR19557-4]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control ab172730 is also shown.

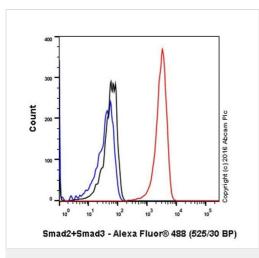
Additional screenshots of mapped reads can be downloaded <u>here</u>. The University of Geneva owns patents relevant to ChlC (Chromatin Immuno-Cleavage) methods.



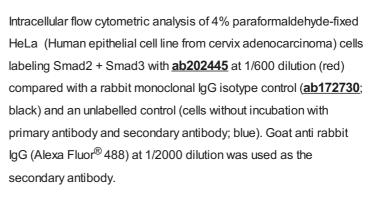
ChIP - Anti-Smad2 + Smad3 antibody [EPR19557-4] - BSA and Azide free (ab232326)

Chromatin was prepared from HaCaT (Human keratinocyte cell line) cells treated with 7ng/ml TGF-β for 1h and non-treated according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25μg of chromatin, 2μg of ab202445 (blue), and 20μl of Anti rabbit lgG sepharose beads. 2μg of rabbit normal lgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

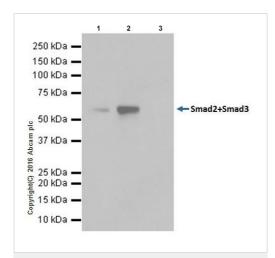
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab202445).



Flow Cytometry (Intracellular) - Anti-Smad2 + Smad3 antibody [EPR19557-4] - BSA and Azide free (ab232326)



This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab202445).



Immunoprecipitation - Anti-Smad2 + Smad3 antibody [EPR19557-4] - BSA and Azide free (ab232326)

Smad2 + Smad3 was immunoprecipitated from 0.35mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with <u>ab202445</u> at 1/40 dilution. Western blot was performed from the immunoprecipitate using <u>ab202445</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

Lane 1: HeLa whole cell lysate 10µg (Input).

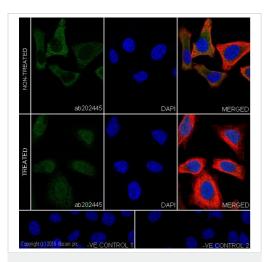
Lane 2: ab202445 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab202445</u> in HeLa whole cell lysate.

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

Exposure time: 3 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab202445).



Immunocytochemistry/ Immunofluorescence - Anti-Smad2 + Smad3 antibody [EPR19557-4] - BSA and Azide free (ab232326)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Smad2 + Smad3 with <a href="mailto:ab202445">ab202445</a> at 1/200 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488) (<a href="mailto:ab150077">ab150077</a>) secondary antibody at 1/1000 dilution (green). The results show signal translocation after TGF-beta (10ng/ml, 1h) treatment on HeLa cells. The nuclear counter stain is DAPI (blue).

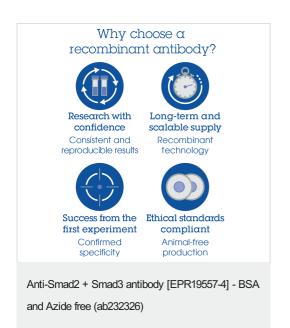
Tubulin is detected with Anti-alpha Tubulin mouse MAb (<u>ab7291</u>) at 1/1000 dilution followed by Goat Anti-Mouse IgG H&L (Alexa Fluor<sup>®</sup> 594) (<u>ab150120</u>) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1:  $\underline{ab202445}$  at 1/200 dilution, followed by Goat Anti-Mouse lgG H&L (Alexa Fluor® 594) ( $\underline{ab150120}$ ) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (<u>ab7291</u>) at 1/1000 dilution,followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) (<u>ab150077</u>) 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 (ab202445).



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