

## Product datasheet

# Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade ab202445

Recombinant RabMAb

★★★★☆ **1 Abreviews** **36 References** [10 Images](#)

### Overview

|                            |  |
|----------------------------|--|
| <b>Product name</b>        | Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade  |
| <b>Description</b>         | Rabbit monoclonal [EPR19557-4] to Smad2 + Smad3 - ChIP Grade   |
| <b>Host species</b>        | Rabbit   |
| <b>Tested applications</b> | <b>Suitable for:</b> Flow Cyt (Intra), ChIC/CUT&RUN-seq, WB, ICC/IF, IP, ChIP  |
| <b>Species reactivity</b>  | <b>Reacts with:</b> Mouse, Rat, Human  |
| <b>Immunogen</b>           | Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.   |
| <b>Positive control</b>    | WB: Recombinant protein fragment human Smad2 and recombinant protein fragment human Smad3; HEK-293, HepG2, HeLa, Jurkat and C6 whole cell lysates; human fetal heart and fetal kidney lysates; mouse brain and heart lysates; rat brain and spleen lysates. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells. IP: HeLa whole cell lysate. ChIP: Chromatin from HaCaT cells treated with 7ng/ml TGF- $\beta$ for 1h. ChIC/CUT&RUN seq: HaCaT cell   |
| <b>General notes</b>       | <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"><li>- High batch-to-batch consistency and reproducibility</li><li>- Improved sensitivity and specificity</li><li>- Long-term security of supply</li><li>- Animal-free production</li></ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p> |

### Properties

|                             |   |
|-----------------------------|---|
| <b>Form</b>                 | Liquid  |
| <b>Storage instructions</b> | 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. |
| <b>Storage buffer</b>       | pH: 7.2<br>Preservative: 0.01% Sodium azide<br>Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA               |
| <b>Purity</b>               | Protein A purified  |

|                     |            |
|---------------------|------------|
| <b>Clonality</b>    | Monoclonal |
| <b>Clone number</b> | EPR19557-4 |
| <b>Isotype</b>      | IgG        |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab202445 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

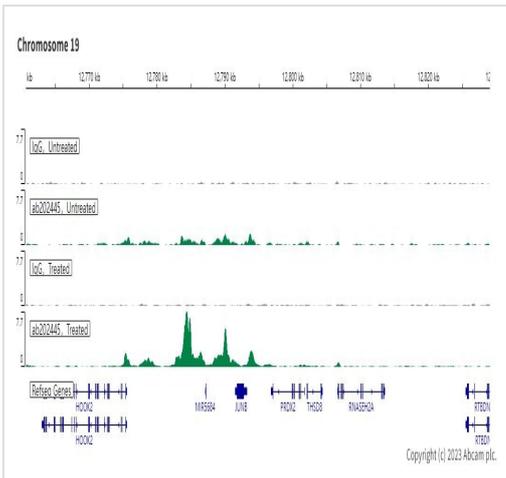
| Application      | Abreviews | Notes   |
|------------------|-----------|---|
| Flow Cyt (Intra) |           | 1/600.  |
| ChIC/CUT&RUN-seq |           | Use at an assay dependent concentration.  |
| WB               |           | 1/1000. Detects a band of approximately 58-62 kDa (predicted molecular weight: 52 kDa). |
| ICC/IF           |           | 1/200.  |
| IP               |           | 1/40.   |
| ChIP             |           | Use 2 µg for 25 µg of chromatin.  |

## Target

**Relevance** 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.

**Cellular localization** Cytoplasm. Nucleus. Note: Cytoplasmic in the absence of ligand. Migrates to the nucleus when complexed with SMAD4.

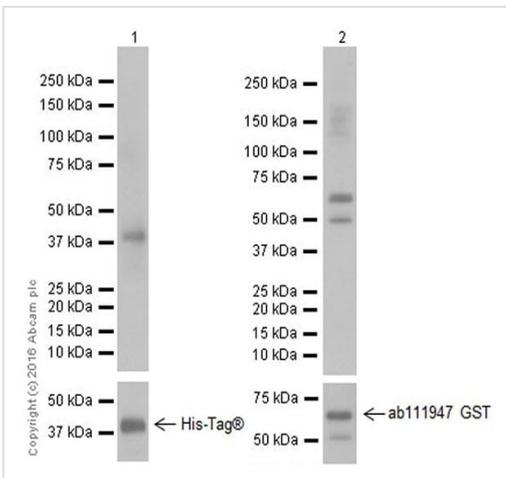
## Images



ChIC/CUT&RUN sequencing - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

ChIC/CUT&RUN was performed using ta pAG-MNase at a final concentration of 700 ng/μL, 2.5 x 10<sup>5</sup> HaCaT (Human keratinocyte cell line) cells (treated with 7ng/ml TGF-β for 1h) and 5 μg 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 [here](#). The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Western blot - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

**All lanes** : Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445) at 1/1000 dilution

**Lane 1** : Recombinant protein fragment human Smad2

**Lane 2** : Recombinant protein fragment human Smad3

Lysates/proteins at 0.01 μg per lane.

**Secondary**

**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

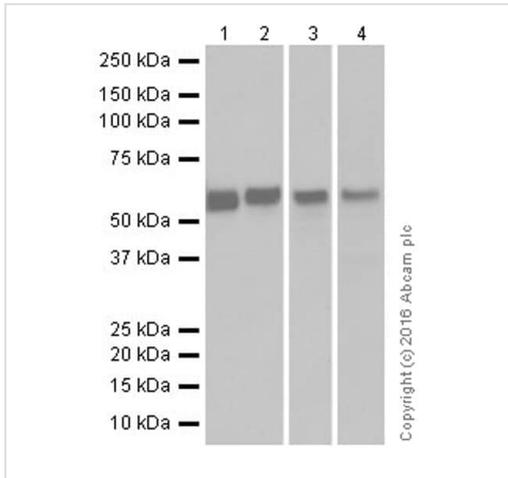
**Predicted band size:** 52 kDa

**Observed band size:** 38,60 kDa

**Exposure time:** 1 second

Blocking/Dilution buffer: 5% NFDm/TBST.

Human Smad2 fragment recombinant protein contains aa2-270 with His-Tag®. Human Smad3 fragment recombinant protein contains aa2-227 with His-Tag® and GST-tag.



Western blot - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

**All lanes** : Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445) at 1/2000 dilution

**Lane 1** : 293 (Human epithelial cell line from embryonic kidney) whole cell lysate

**Lane 2** : HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

**Lane 3** : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 4** : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lysates/proteins at 10 µg per lane.

### Secondary

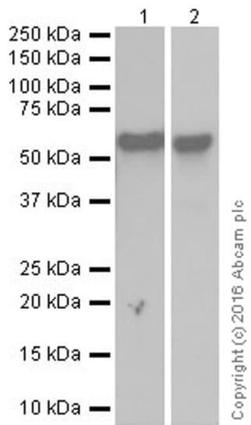
**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

**Predicted band size:** 52 kDa

**Observed band size:** 58-62 kDa

Blocking/Dilution buffer: 5% NFDm/TBST.

Exposure time: Lane 1/2: 10 seconds; Lane 3: 3 seconds; Lane 4: 1 seconds.



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Western blot - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

**All lanes** : Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445) at 1/2000 dilution

**Lane 1** : Human fetal heart lysate

**Lane 2** : Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

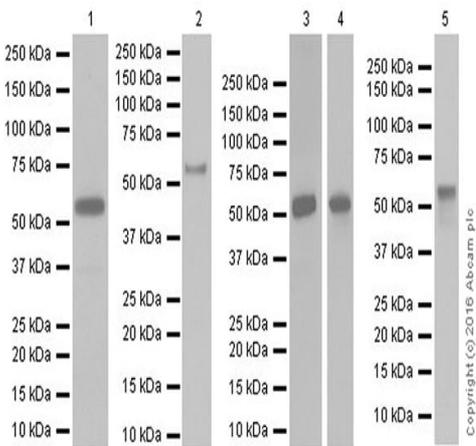
**All lanes** : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

**Predicted band size:** 52 kDa

**Observed band size:** 58-62 kDa

Blocking/Dilution buffer: 5% NFDm/TBST.

Exposure time: Lane 1: 30 seconds; Lane 2: 10 seconds.



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Western blot - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

**Lane 1** : Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445) at 1/1000 dilution

**Lanes 2 & 5** : Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445) at 1/2000 dilution

**Lanes 3-4** : Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445) at 1/20000 dilution

**Lane 1** : Mouse brain lysate

**Lane 2** : Mouse heart lysate

**Lane 3** : Rat brain lysate

**Lane 4** : C6 (Rat glial tumor cell line) whole cell lysate

**Lane 5** : Rat spleen lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

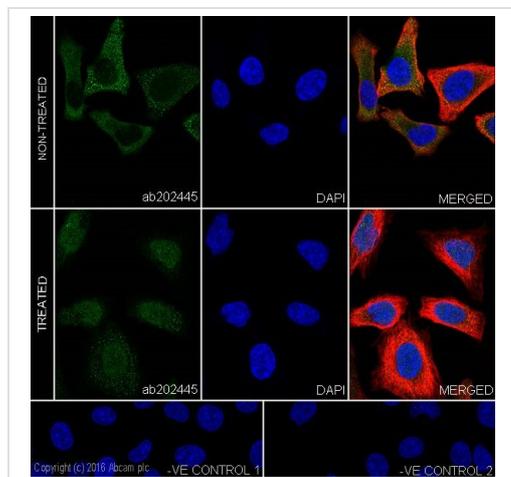
**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

**Predicted band size:** 52 kDa

**Observed band size:** 58-62 kDa

Blocking/Dilution buffer: 5% NFDm/TBST.

Exposure time: Lane 1: 3 minutes; Lane 2: 10 seconds; Lane 3: 30 seconds; Lane 4: 3 seconds; Lane 5: 5 seconds.



Immunocytochemistry/ Immunofluorescence - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

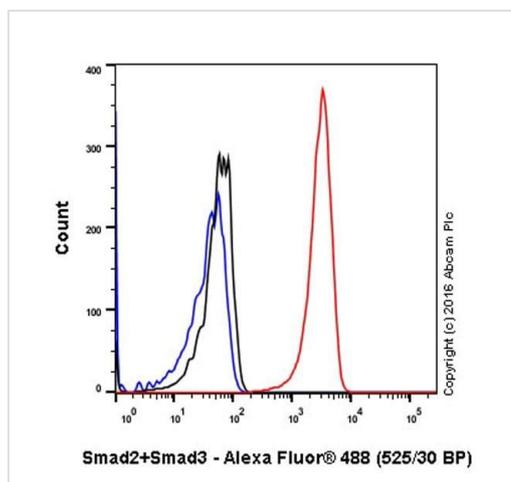
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Smad2 + Smad3 with ab202445 at 1/200 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488) (**ab150077**) secondary antibody 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 (**ab7291**) at 1/1000 dilution followed by Goat Anti-Mouse IgG H&L (Alexa Fluor<sup>®</sup> 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

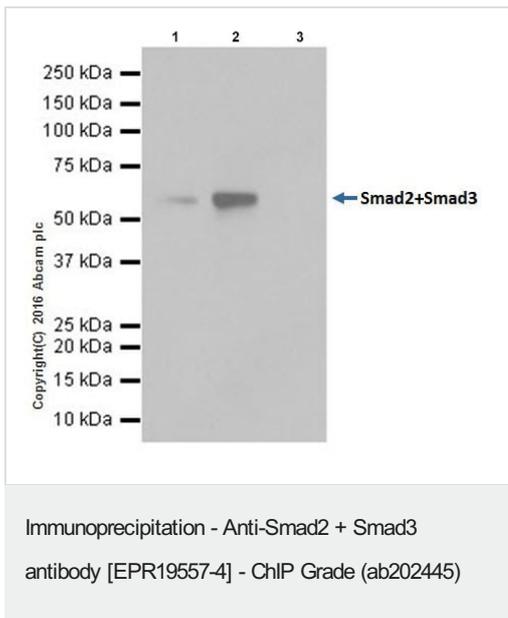
-ve control 1: ab202445 at 1/200 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor<sup>®</sup> 594) (**ab150120**) secondary antibody at 1/1000 dilution.

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



Flow Cytometry (Intracellular) - Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP Grade (ab202445)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Smad2 + Smad3 with ab202445 at 1/600 dilution (red) compared with a rabbit monoclonal IgG isotype control (**ab172730**; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (Alexa Fluor<sup>®</sup> 488) at 1/2000 dilution was used as the secondary antibody.



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

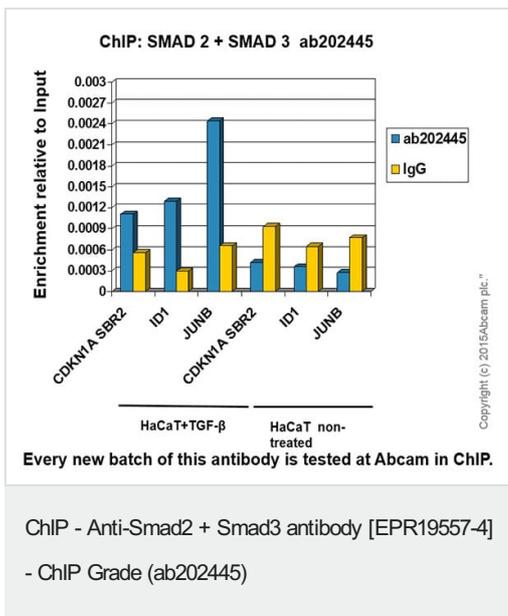
Lane 1: HeLa whole cell lysate 10µg (Input).

Lane 2: ab202445 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG ([ab172730](#)) instead of ab202445 in HeLa whole cell lysate.

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

Exposure time: 3 seconds.



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 IgG sepharose beads. 2µg of rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-Smad2 + Smad3 antibody [EPR19557-4] - ChIP  
Grade (ab202445)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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