abcam

Product datasheet

Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] ab254407

重组 RabMAb

6 References 10 图像

概述

产品名称	Anti-Smad2 (phospho T8) + Smad3 (phospho T8)抗体[EPR23682-64]
描述	兔单克隆抗体[EPR23682-64] to Smad2 (phospho T8) + Smad3 (phospho T8)
宿主	Rabbit
经测试应用	**适用于:** ICC/IF, Dot blot, IP, ChIC/CUT&RUN-seq, WB **不适用于:** Flow Cyt or IHC-P
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	**This product was produced with the following immunogens:** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: RAW264.7, PC-12, HaCaT, and HaCaT (treated with 100nM calyculin A for 30 min) whole cell lysates; Human liver cancer tissue lysate; Mouse lung and liver tissue lysates. ICC/IF: HaCaT and RAW264.7 cells. IP: HaCaT whole cell lysate. ChIC/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	Preservative: 0.01% Sodium azide Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

纯度	Protein A purified
克隆	单克隆
克隆编号	EPR23682-64
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab254407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。

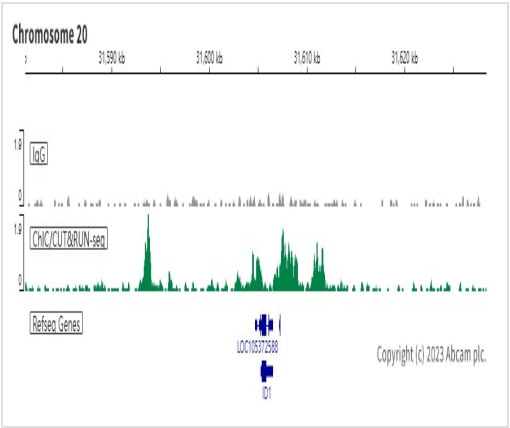
应用	Ab评论	说明
ICC/IF		1/50.
Dot blot		1/1000.
IP		1/30.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg
WB		1/1000. Detects a band of approximately 55, 60 kDa.

应用说明 Is unsuitable for Flow Cyt or IHC-P.

靶标

细胞定位 Smad2: 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. Smad3: 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

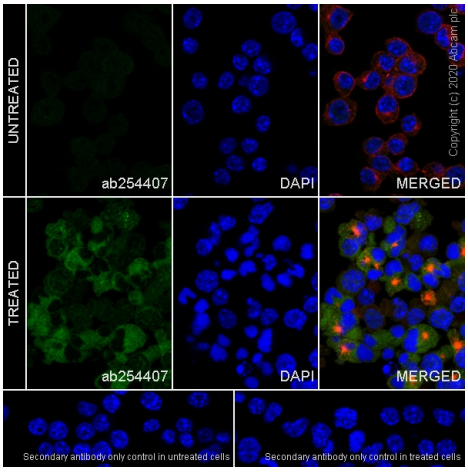


ChIC/CUT&RUN sequencing - Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] (ab254407)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2 x 10^5 HaCaT (human skin keratinocyte) cells and 5µg of ab254407 [EPR23682-64]. 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**.

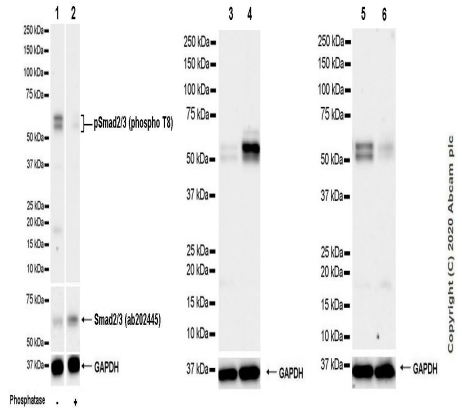
The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Immunocytochemistry/ Immunofluorescence - Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] (ab254407)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 cells labelling Smad2 (phospho T8) + Smad3 (phospho T8) with ab254407 at 1/50 (9.34 ug/ml) dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing weak cytoplasmic and nuclear staining in RAW 264.7 cells, while strong cytoplasmic and weak nuclear staining in RAW 264.7 cells treated with calyculin A (100 nM) for 30 mins is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Western blot - Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] (ab254407)

All lanes : Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] (ab254407) at 1/1000 dilution

Lane 1 : HaCaT (human skin keratinocyte), whole cell lysate at 10 µg
Lane 2 : HaCaT - phosphatase treated membrane, whole cell lysate at 10 µg
Lanes 3 & 5 : HaCaT whole cell lysate at 20 µg
Lane 4 : HaCaT treated with 100nM calycin A for 30 min, whole cell lysate at 20 µg
Lane 6 : HaCaT treated with /ml TGF beta1 for 24h, whole cell lysate at 20 µg

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

Observed band size: 55,60 kDa

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

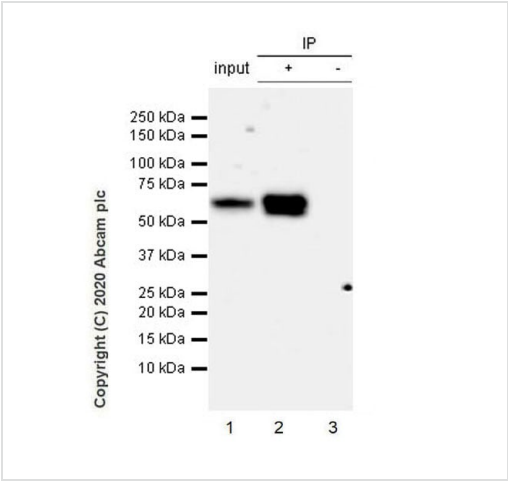
The molecular weight observed is consistent with what has been described in the literature. (PMID: 25998442, 30666129).

Calyculin A is a known phosphatase inhibitor, which increased the level of pSmad2/3 (T8). The shifted band after treated with Calyculin A might be due to multiple phosphorylation events.

The down-regulation of pSmad2 (T8) is induced by TGF-beta1 treatment in HaCaT (PMID: 19201832).

Bands between 15-25kDa may be caused by degradation.

Exposure time: Lane 1, 2: 26 secondsLane 3, 4: 59 secondsLane 5, 6: 3 minutes



Immunoprecipitation - Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] (ab254407)

Smad2 (phospho T8) + Smad3 (phospho T8) was immunoprecipitated from 0.35 mg HaCaT (human skin keratinocyte), whole cell lysate with ab254407 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab254407 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: HaCaT (human skin keratinocyte), whole cell lysate 10 ug

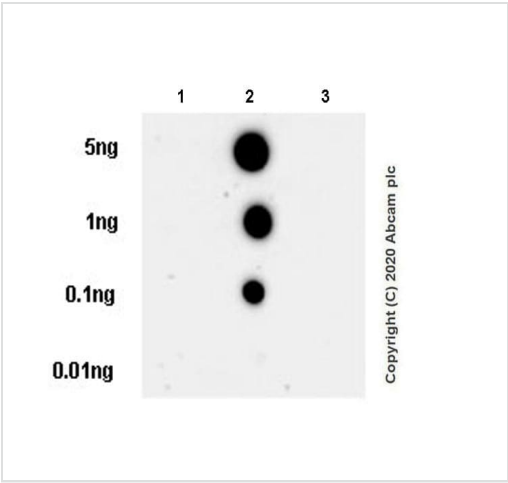
Lane 2: ab254407 IP in HaCaT whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab254407 in HaCaT whole cell lysate

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

Exposure time: 110 seconds

The molecular weight observed is consistent with what has been described in the literature. (PMID: 25998442, 30666129).



Dot Blot - Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] (ab254407)

Dot blot analysis of Smad2 (phospho T8) + Smad3 (phospho T8) using ab254407 at 1/1000 (0.467 ug/ml) dilution followed by a Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100,000 dilution.

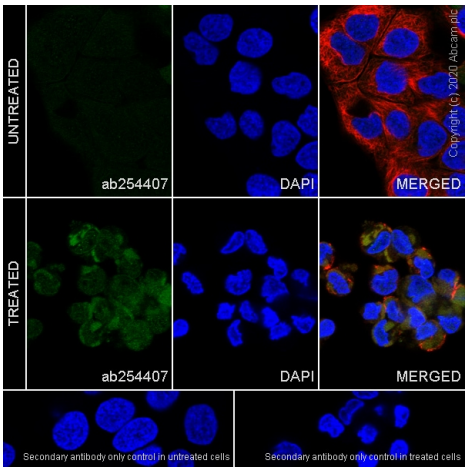
Exposure time: 3 minutes.

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

Lane 1: Smad2/3 peptide (aa 6-14)

Lane 2: Smad2/3 (phospho T8) peptide (aa 2-10)

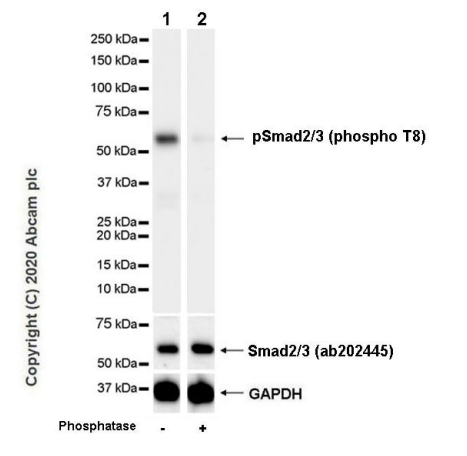
Lane 3: Smad2/3 peptide (aa 2-14)



Immunocytochemistry/ Immunofluorescence - Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] (ab254407)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HaCaT cells labelling Smad2 (phospho T8) + Smad3 (phospho T8) with ab254407 at 1/50 (9.34 ug/ml) dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing weak cytoplasmic and nuclear staining in HaCaT cells, while strong cytoplasmic and weak nuclear staining in HaCaT cells treated with calyculin A (100 nM) for 30 mins is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Western blot - Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] (ab254407)

All lanes : Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] (ab254407) at 1/1000 dilution

Lane 1 : RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage), whole cell lysate
Lane 2 : RAW264.7 - phosphatase treated membrane, whole cell lysate

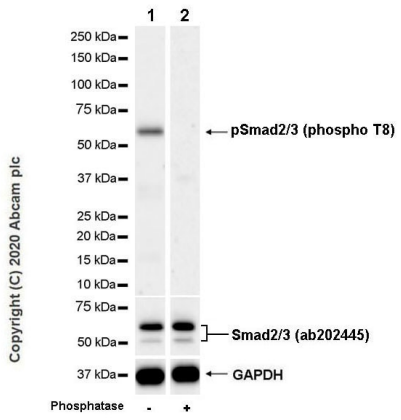
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Observed band size: 60 kDa

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

Exposure time: 26 seconds.



Western blot - Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] (ab254407)

All lanes : Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] (ab254407) at 1/1000 dilution

Lane 1 : PC-12 (rat adrenal gland pheochromocytoma), whole cell lysate
Lane 2 : PC-12 - phosphatase treated membrane, whole cell lysate

Lysates/proteins at 10 µg per lane.

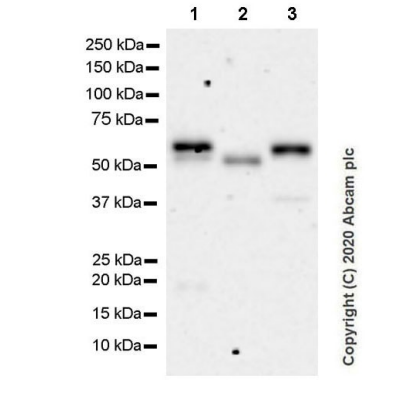
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Observed band size: 60 kDa

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

The molecular weight observed is consistent with what has been described in the literature. (PMID: 25998442, 30666129)

Exposure time: 59 seconds.



Western blot - Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] (ab254407)

All lanes : Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] (ab254407) at 1/1000 dilution

Lane 1 : Human liver cancer tissue lysate
Lane 2 : Mouse lung tissue lysate
Lane 3 : Mouse liver tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

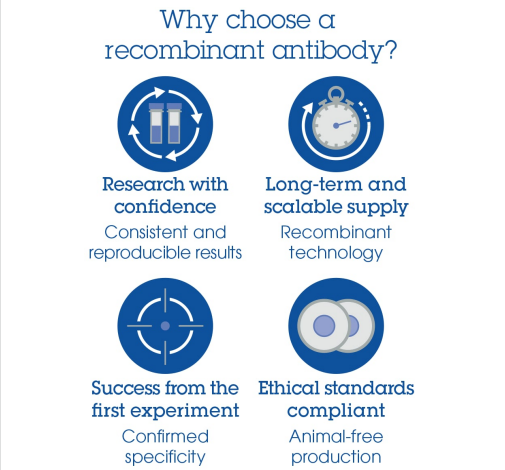
Observed band size: 55,60 kDa

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

The molecular weight observed is consistent with what has been described in the literature. (PMID: 25998442, 30666129).

Bands between 15-25kDa may be caused by degradation.

Exposure time: 3 minutes.



Anti-Smad2 (phospho T8) + Smad3 (phospho T8) antibody [EPR23682-64] (ab254407)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.cn/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors