abcam

ab40855

Anti-Smad2 antibody [EP784Y]

Anti-Smad2 antibody [EP784Y] (ab40855) is a rabbit monoclonal antibody detecting Smad2 in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**. Suitable for **Human, Rat**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 100 publications
- Trusted since 2006

Recombinant | RabMAb | Advanced Validation | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EP784Y
Purification technique	Affinity purification Protein A
Specificity	This antibody is specific for MH 1 domain of Smad2.
Concentration	0.5 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IP

Tested

Species	Human
Dilution info	1/20 - 1/50
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Rat
Dilution info	1/2000 - 1/10000
Notes	-

Species	Human
Dilution info	1/2000 - 1/10000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/250
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/20 - 1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/50
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information SMAD2

Function	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. Promotes TGFB1-mediated transcription of odontoblastic differentiation genes in dental papilla cells (By similarity). Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator. May act as a tumor suppressor in colorectal carcinoma (PubMed:8752209).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-Smad2 antibody [EP784Y] (ab40855) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human, Rat samples.

What is the molecular weight of Smad2?
Anti-Smad2 [EP784Y] (ab40855) specifically detects a band for Smad2 (UniProt: Q15796) at a molecular weight of 58kDa.

Trusted by the scientific community
Anti-Smad2 [EP784Y] (ab40855) was first used in a scientific publication in 2006 and has been cited over 100 times in peer-reviewed journals.

Specificity confirmed
The specificity of Anti-Smad2 antibody [EP784Y] (ab40855) has been confirmed by Western blot testing in SMAD2 Knockout HeLa cells.

Other related products
We have a range of other formats of antibody clone [EP784Y] also available for your convenience: ab40855, Carrier free - ab157371, PE - ab212096, Alexa Fluor® 488 - ab215913

Species reactivity
Mouse: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Smad2 also known as Mothers against decapentaplegic homolog 2 (MAD2) or MADR2 is a signaling protein involved in the transforming growth factor-beta (TGF-B) receptor pathway. Smad2 has a molecular mass of approximately 58 kDa and expresses in various tissues including epithelial mesenchymal and endothelial cells. Smad2 undergoes phosphorylation on serine residues in response to TGF-B signaling converting it into phosphorylated forms often referred to as p-Smad2 or phospho-Smad2. These phosphorylated forms are critical for the relay of signals from the cell surface to the nucleus.
Biological function summary	Smad2 acts as an intracellular mediator for TGF-B signaling a pathway important for regulating cell proliferation differentiation and apoptosis. Smad2 typically functions as part of a heteromeric complex with Smad4 another key player in TGF-B signaling. Upon activation phosphorylated Smad2 combines with Smad4 to form

	a complex that translocates into the nucleus. This complex then binds to specific DNA sequences to regulate the transcription of target genes involved in processes such as cell growth inhibition and extracellular matrix production.
Pathways	Smad2 is integral to the TGF-B and activin receptor signaling pathways. These pathways are essential in controlling cell growth and immune responses. Smad2 interacts with other proteins such as Smad3 in addition to Smad4 to modulate gene expression effectively. The interaction between Smad2 and these proteins ensures precise control of cellular responses to external stimuli emphasizing its pivotal role in maintaining cellular homeostasis.
Associated diseases and disorders	Smad2 correlates with various pathological conditions including fibrosis and cancer. Aberrant Smad2 signaling can contribute to the development of these diseases as excessive TGF-B signaling promotes fibrotic tissue deposition and tumor progression. Smad2 connects with other proteins like Smad3 in these pathological contexts both acting as mediators of abnormal cellular behaviors. Understanding the regulatory mechanisms of Smad2 can help develop therapeutic strategies against disorders linked to dysregulated TGF-B signaling.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

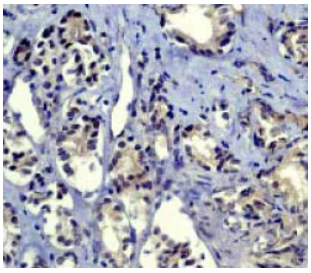
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

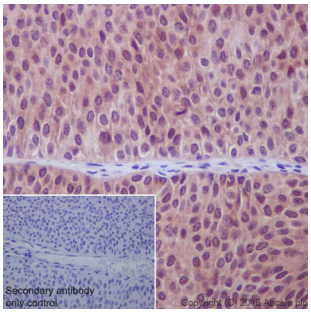
16 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad2 antibody [EP784Y] (ab40855)

Smad2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Smad2 antibody

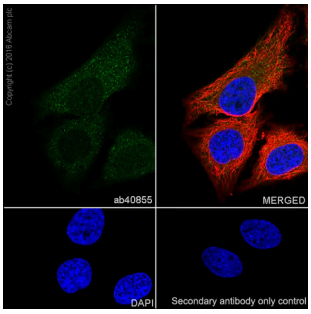
ab40855 at a 1:100 dilution staining Smad2 in human prostate carcinoma tissue.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad2 antibody [EP784Y] (ab40855)

Smad2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Smad2 antibody

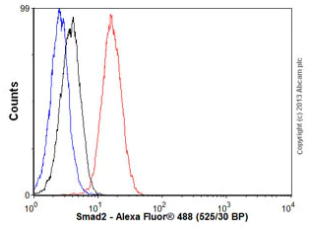
ab40855 stainingSmad2 in human bladder carcinoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/50. A ImmunoHistoProbe one step HRP Polymer was used as a secondary antibody, ready to use.
Negative control 1: PBS in place of primary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Smad2 antibody [EP784Y] (ab40855)

Smad2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Smad2 antibody

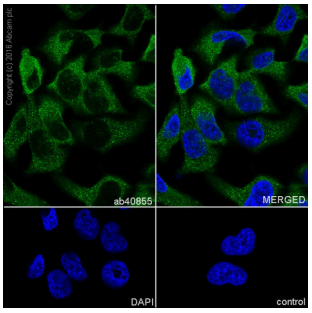
ab40855 staining Smad2 in HeLa (human cervix adenocarcinoma) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody at a dilution of 1/1000. ab195889 was used as a counterstain for primary antibody ab40855 at 1/1000. DAPI was used as a nuclear counterstain and PBS as a negative control.



Flow Cytometry (Intracellular) - Anti-Smad2 antibody [EP784Y] (ab40855)

Smad2 Flow Cytometry (Intracellular) staining using rabbit Anti-Smad2 antibody

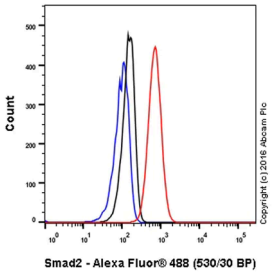
Overlay histogram showing PC3 cells stained with ab40855 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab40855, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Immunocytochemistry/ Immunofluorescence - Anti-Smad2 antibody [EP784Y] (ab40855)

Smad2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Smad2 antibody

Immunofluorescence staining of HeLa cells with purified ab40855 at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was an Alexa Fluor® 488 conjugated goat anti-rabbit (ab150077), used at a dilution of 1/1000. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative control is shown in bottom right hand panel - for the negative control, PBS was used instead of the primary antibody.

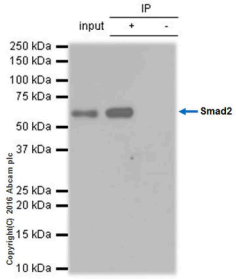


Flow Cytometry (Intracellular) - Anti-Smad2 antibody [EP784Y] (ab40855)

Smad2 Flow Cytometry (Intracellular) staining using rabbit Anti-Smad2 antibody

ab40855 staining Smad2in the human cell line HeLa (Human epithelial cell line from cervix adenocarcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/20. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.
Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Immunoprecipitation - Anti-Smad2 antibody [EP784Y] (ab40855)

ab40855 (purified) at 1/20 immunoprecipitating EGFR in HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate.
Lane 1 (input): HeLa whole cell lysate (10µg)

Lane 2 (+): ab40855 + HeLa whole cell lysate.

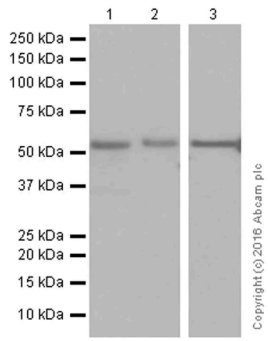
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab40855 in HeLa whole cell lysate.

For western blotting, ab131366 VeriBlot for IP (HRP) was used for detection (1/1000).

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

All lanes:
Immunoprecipitation - Anti-Smad2 antibody [EP784Y] (ab40855)

Predicted band size: 52 kDa



Western blot - Anti-Smad2 antibody [EP784Y] (ab40855)

Diluting and blocking buffer: 5% NFDM /TBST
All lanes:
Western blot - Anti-Smad2 antibody [EP784Y] (ab40855) at 1/2000 dilution

Lane 1:
A-673 (Human muscle Ewing's Sarcoma cell line) whole cell lysate at 20 µg

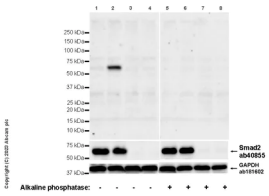
Lane 2:
HUVEC (Human umbilical vein endothelial cell line) whole cell lysate at 20 µg

Lane 3:
C6 (Rat glial tumor cell line) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 52 kDa



Western blot - Anti-Smad2 antibody [EP784Y] (ab40855)

Smad2 Western blot staining using rabbit Anti-Smad2 antibody

The molecular weight observed is consistent with what has been described in the literature (PMID: 34655614).

The up-regulation of pSmad2 is induced by TGF-beta treatment (PMID: 34655614, 24347165).

In Western blot, ab316117 was shown to bind specifically to Smad2. Target of interest was observed at 60 kDa in wild-type Hela cell lysates (lanes 2) with no signal observed at this size in Smad2 knockout cell line lysates (lanes 3-4 KO cell line ab255430/KO cell lysate ab263833).

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

In Western blot, Anti-Smad2 antibody [EP784Y] - Total protein control (ab40855) staining at 1/1000 dilution.

All lanes:
Western blot - Anti-Smad2 (phospho S465 + S467) antibody [EPR28610-44] (ab316117) at 1/1000 dilution

Lane 1:
Untreated wild-type HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate (untreated membrane) at 20 µg

Lane 2:
Wild-type Hela treated with 20 ng/ml TGF beta 1 for 60 minutes, whole cell lysate (untreated membrane) at 20 µg

Lane 3:
Untreated Smad2 knockout Hela whole cell lysate (untreated membrane) at 20 µg

Lane 4:
Smad2 knockout Hela treated with 20 ng/ml TGF beta 1 for 60 minutes, whole cell lysate (untreated membrane) at 20 µg

Lane 5:
Untreated wild-type HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

Lane 6:
Wild-type Hela treated with 20 ng/ml TGF beta 1 for 60 minutes, whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

Lane 7:
Untreated Smad2 knockout Hela whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

Lane 8:

Smad2 knockout Hela treated with 20 ng/ml TGF beta 1 for 60 minutes, whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

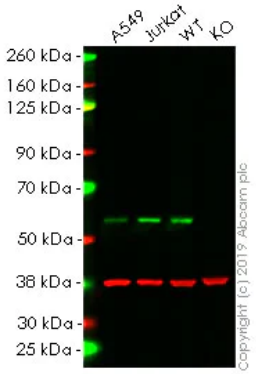
Secondary

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

Performed under reducing conditions.

Observed band size: 60 kDa, 36 kDa

Exposure time: 37s



Western blot - Anti-Smad2 antibody [EP784Y] (ab40855)

Smad2 Western blot staining using rabbit Anti-Smad2 antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab40855 observed at 58 kDa. Red - loading control, ab8245 observed at 37 kDa.
ab40855 was shown to react with Smad2 in wild-type HeLa. Loss of signal was observed when knockout cell line ab255430 (knockout cell lysate ab263833) was used. Wild-type and Smad2 knockout samples were subjected to SDS-PAGE. ab40855 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 2000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Smad2 antibody [EP784Y] (ab40855) at 1/2000 dilution

Lane 1:
A549 cell lysate at 20 µg

Lane 2:
Jurkat cell lysate at 20 µg

Lane 2:
Western blot - Human SMAD2 knockout HeLa cell line (ab255430)

Lane 3:
Wild-type HeLa cell lysate at 20 µg

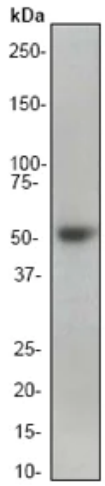
Lane 4:
SMAD2 knockout HeLa cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Predicted band size: 52 kDa

Observed band size: 37 kDa, 55 kDa



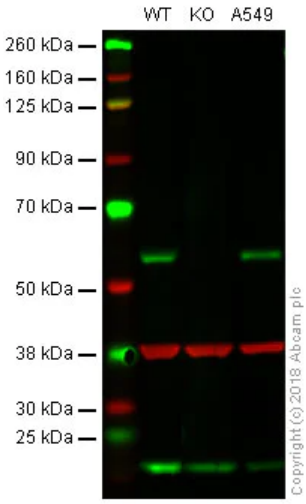
Western blot - Anti-Smad2 antibody [EP784Y] (ab40855)

All lanes:
Western blot - Anti-Smad2 antibody [EP784Y] (ab40855) at 1/500000 dilution

All lanes:
Jurkat cell lysate

Predicted band size: 52 kDa

Observed band size: 58 kDa



Western blot - Anti-Smad2 antibody [EP784Y] (ab40855)

Lanes 1 - 3: Merged signal (red and green). Green - ab40855 observed at 58 kDa. Red - loading control, ab9484, observed at 37 kDa.

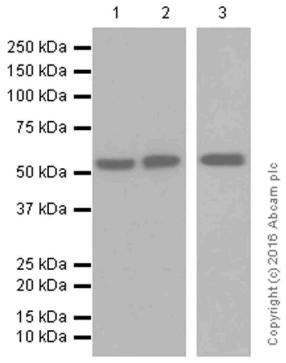
ab40855 was shown to specifically react with Smad2 in wild-type HeLa cells as signal was lost in Smad2 knockout cells. Wild-type and SMAD2 knockout samples were subjected to SDS-PAGE. ab40855 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/10000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.
All lanes:
Western blot - Anti-Smad2 antibody [EP784Y] (ab40855) at 1/1000 dilution

Lane 1:
Wild-type HeLa whole cell lysate at 20 µg

Lane 2:
Smad2 knockout HeLa whole cell lysate at 20 µg

Lane 3:
A549 whole cell lysate at 20 µg

Predicted band size: 52 kDa



Western blot - Anti-Smad2 antibody [EP784Y] (ab40855)

Blocking and diluting buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-Smad2 antibody [EP784Y] (ab40855) at 1/10000 dilution

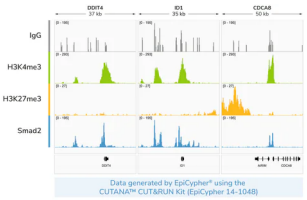
Lane 1:
Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate at 20 µg

Lanes 2 - 3:
HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

Secondary

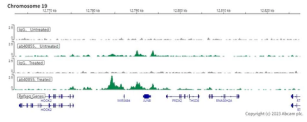
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 20000 µg

Predicted band size: 52 kDa



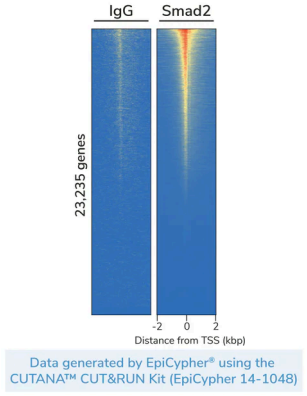
ChIC/CUT&RUN sequencing - Anti-Smad2 antibody [EP784Y] (ab40855)

CUT&RUN profiling with Smad2 antibody reveals the expected genomic enrichment pattern in cells. Representative genome browser tracks show CUT&RUN data generated using the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048) with Smad2 antibody (Abcam ab40855, 0.5 µg). 500,000 HeLa cells were used per reaction. IgG, H3K4me3, and H3K27me3 antibodies were included as controls to assess non-specific background, active promoters, and repressed chromatin, respectively. Libraries were prepared using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001). Sequencing was performed with paired-end 50 bp reads, and data were processed on CUTANA™ Cloud (cloud.epicypher.com) by alignment to the hg38 genome. Images were generated using Integrative Genomics Viewer (IGV, Broad Institute).



ChIC/CUT&RUN sequencing - Anti-Smad2 antibody [EP784Y] (ab40855)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 HaCaT (Human keratinocyte cell line) cells (treated with 7ng/ml TGF-β for 1h) and 5 µg of ab40855 [EP784Y]. 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 found in the Protocol booklet in the Product Protocol section. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



ChIC/CUT&RUN sequencing - Anti-Smad2 antibody [EP784Y] (ab40855)

CUT&RUN profiling with Smad2 antibody demonstrates robust genome-wide enrichment in cells. Heatmaps of genome-wide signal flanking annotated transcription start sites (TSSs, +/- 2 kbp) display CUT&RUN data generated using the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048) with Smad2 antibody (Abcam ab40855, 0.5 µg). 500,000 HeLa cells were used per reaction. IgG antibody was included as a negative control to assess non-specific background. Libraries were prepared using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001). Sequencing was performed with paired-end 50 bp reads, and data were processed on CUTANA™ Cloud (cloud.epicypher.com) by alignment to the hg38 genome. Heatmaps were generated using deepTools (Ramírez et al., Nucleic Acids Res. 2014; PMID: 24799436). Row-linked data are ranked by intensity relative to Smad2, with red indicating high localized enrichment and blue denoting background.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.