abcam

ab40854

Anti-Smad3 antibody [EP568Y]

Anti-Smad3 antibody [EP568Y] (ab40854) is a rabbit monoclonal antibody detecting Smad3 in **Western Blot, Flow Cytometry (Intra), ICC/IF, ELISA**. Suitable for **Human, Rat**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 300 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	EP568Y
Purification technique	Affinity purification Protein A
Concentration	1.127 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Not recommended

Species	Rat, Human, Mouse, Chicken, Pig, Synthetic peptide - Human
Dilution info	-
Notes	-

WB

Tested

Species	Rat
Dilution info	1/1000 - 1/10000
Notes	-

Species	Human
Dilution info	1/1000 - 1/10000
Notes	-

Predicted

Species	Mouse, Chicken, Pig
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

sELISA

Tested

Species	Synthetic peptide - Human
Dilution info	0.5 µg/mL
Notes	For sandwich ELISA, use this antibody as Detection at 0.5 µg/ml with Mouse monoclonal [AF9F7] to Smad3 (ab75512) as Capture.

Expected

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

Predicted

Species	Mouse, Chicken, Pig
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500 - 1/2000
Notes	-

Expected

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

Predicted

Species	Mouse, Chicken, Pig
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-

Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/50 - 1/210
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	-

Predicted

Species	Mouse, Chicken, Pig
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human, Mouse, Rat, Chicken, Pig, Synthetic peptide - Human
Dilution info	-
Notes	-

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	-
Notes	-

Expected

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

Predicted

Species	Mouse, Chicken, Pig
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Target data

See full target information SMAD3

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 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.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
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-Smad3 antibody [EP568Y] (ab40854) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunocytochemistry/immunofluorescence (ICC/IF), ELISA in Human, Rat samples.

What is the molecular weight of Smad3?
Anti-Smad3 [EP568Y] (ab40854) specifically detects a band for Smad3 (UniProt: P84022) at a molecular weight of 48kDa.

Trusted by the scientific community
Anti-Smad3 [EP568Y] (ab40854) was first used in a scientific publication in 2006 and has been cited over 300 times in peer-reviewed journals.

Reviewed by scientists
Anti-Smad3 [EP568Y] (ab40854) has over 10 independent reviews from customers.

Specificity confirmed
The specificity of Anti-Smad3 antibody [EP568Y] (ab40854) has been confirmed by Western blot testing in SMAD3 Knockout A549 cells.

Other related products
We have a range of other formats of antibody clone [EP568Y] also available for your convenience: ab40854, Carrier free - ab157372, Alexa Fluor® 488 - ab204257, Alexa Fluor® 647 - ab204461, HRP - ab204462, Alexa Fluor® 594 - ab206350, Alexa Fluor® 555 - ab206582, PE - ab208751

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.

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.

Supplementary info

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

Activity summary	Smad3 also known as Mothers against decapentaplegic homolog 3 is a protein that plays a mechanical role in signal transduction. It acts mainly as a transcription factor and gets activated through phosphorylation. The molecular weight of Smad3 is approximately 48 kDa. It is expressed widely across numerous tissues including the cellular nucleus where it executes its function after activation.
Biological function summary	Smad3 acts as a mediator of signal transduction for the TGF-beta (transforming growth factor-beta) superfamily forming a complex with phosphorylated Smad2. This enables it to regulate transcriptional activity influencing cell proliferation differentiation and apoptosis. Smad3 also participates in various cellular processes by interacting with other co-factors and regulatory proteins that aid in fine-tuning its function.
Pathways	Smad3 plays an important role in the TGF-beta signaling pathway where it works closely with Smad4 to propagate the signal. Upon phosphorylation it forms a complex with co-Smad (Smad4) and moves into the nucleus to influence gene expression. Smad3 is also involved in pathways related to oncogenesis and tissue fibrosis indicating its significant role in cellular regulation and response mechanisms.
Associated diseases and disorders	Smad3 is associated with fibrotic diseases and cancers particularly in tissues such as the liver and lungs. Altered Smad3 signaling contributes to the pathological process occurring in fibrotic disorders often interacting with Smad4 in these abnormalities. Dysregulated Smad3 expression or mutations can also lead to oncogenic transformations highlighting its critical involvement in disease states.

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

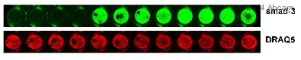
We do not recommend this combination. It is not covered by our product promise.

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

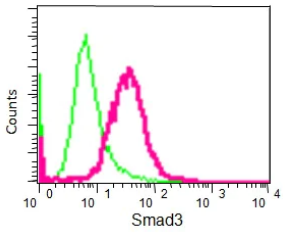


This image is courtesy of a customer review submitted by Francesco Elia Marino

Immunocytochemistry/ Immunofluorescence - Anti-Smad3 antibody [EP568Y] (ab40854)

Smad3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Smad3 antibody

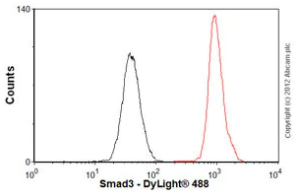
ab40854 staining Smad3 in human granulosa cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with ethanol and triton and blocked for 1 hour at 26°C. Samples were incubated with primary antibody (1/200) for 16 hours at 4°C. An undiluted IRDye® 800CW-conjugated goat anti-rabbit IgG (H+L) polyclonal was used as the secondary antibody. Left - negative control (4 replicates).



Flow Cytometry (Intracellular) - Anti-Smad3 antibody [EP568Y] (ab40854)

Smad3 Flow Cytometry (Intracellular) staining using rabbit Anti-Smad3 antibody

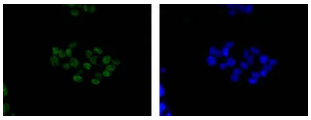
Intracellular Flow Cytometry analysis of HT-29 cells labelling Smad3 with purified ab40854 at 1/210 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Green - Isotype control, rabbit monoclonal IgG.



Flow Cytometry (Intracellular) - Anti-Smad3 antibody [EP568Y] (ab40854)

Smad3 Flow Cytometry (Intracellular) staining using rabbit Anti-Smad3 antibody

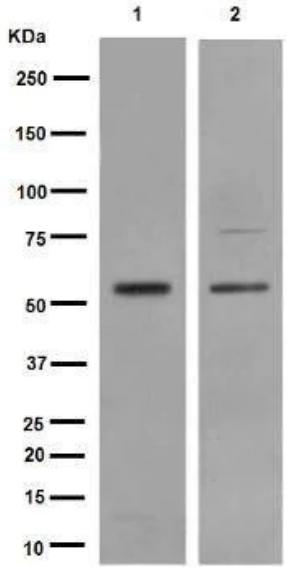
Overlay histogram showing HCT116 cells stained with unpurifiedab40854 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab40854, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 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. Acquisition of >5,000 events was performed.



Immunocytochemistry/ Immunofluorescence - Anti-Smad3 antibody [EP568Y] (ab40854)

Smad3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Smad3 antibody

Immunocytochemsitry/Immunofluorescence analysis of HepG2 cells labelling Smad3 (green) with purified ab40854 at 1/2000 . Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/200) was used as the secondary antibody (green, left panel). Counterstained with DAPI (blue, right panel).



Western blot - Anti-Smad3 antibody [EP568Y] (ab40854)

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

All lanes:
Western blot - Anti-Smad3 antibody [EP568Y] (ab40854) at 1/5300 dilution

Lane 1:
HT-29 cell lysate at 10 µg

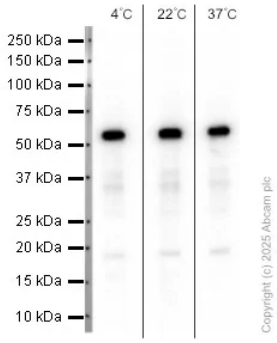
Lane 2:
HT-1080 cell lysate at 10 µg

Secondary

All lanes:
Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 48 kDa

Observed band size: 58 kDa



Western blot - Anti-Smad3 antibody [EP568Y] (ab40854)

Smad3 Western blot staining of HT-29 whole cell lysate using rabbit Anti-Smad3 antibody

This blot was produced using 4-20% SDS-PAGE containing 15 µg of HT-29 whole cell lysate per lane at 150V for 1hr before being transferred onto a 0.45 µm PVDF membrane at 75V for 1hr. The membrane was then blocked for 1hr using 5% NFDM/TBST, then incubated with ab40854 (1/5000) at room temperature for 1hr. After being washed three times in TBST, the membrane was incubated with Peroxidase conjugated goat anti-rabbit IgG (H+L) (ab97051) at 1/20,000 dilution for 1hr at room temperature. The membrane was washed three times again. Then the signal was developed using the ECL technique.

ab40854 was stored at a range of temperatures (+4°C, +22°C, +37°C) for 1 week before being tested in WB. The image shows the band intensity remains relatively constant across all storage temperatures, demonstrating that antibody activity is not affected under these conditions. This data was generated as part of a structured antibody stability study supporting Abcam’ s transition from cold⊠chain to ambient shipping.

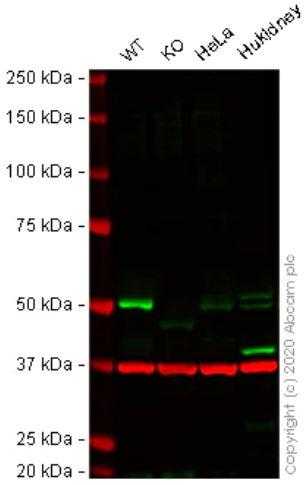
All lanes:
Western blot - Anti-Smad3 antibody [EP568Y] (ab40854) at 1/5000 dilution

All lanes:
HT-29 whole cell lysate at 15 µg with NDFM/TBST

Secondary

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

Exposure time: 10s



Western blot - Anti-Smad3 antibody [EP568Y] (ab40854)

Lanes 1 - 4: Merged signal (red and green). Green - ab40854 observed at 48 kDa. Red - loading control, ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.
ab40854 was shown to react with Smad3 in western blot. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab40854 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Smad3 antibody [EP568Y] (ab40854) at 1/1000 dilution

Lane 1:
Wild-type A549 (Human lung carcinoma cell line) whole cell lysate at 20 µg

Lane 2:
Western blot - Human SMAD3 knockout A549 cell lysate (ab264513)

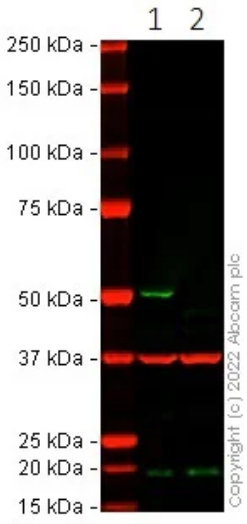
Lane 3:
HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

Lane 4:
Human Kidney cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 48 kDa

Observed band size: 50 kDa



Western blot - Anti-Smad3 antibody [EP568Y] (ab40854)

Smad3 Western blot staining using rabbit Anti-Smad3 antibody

False colour image of Western blot: Anti-Smad3 antibody [EP568Y] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab40854 was shown to bind specifically to Smad3. A band was observed at 50 kDa in wild-type A549 cell lysates with no signal observed at this size in SMAD3 CRISPR-Cas9 edited cell line ab277888 (CRISPR-Cas9 edited cell lysate None). The band observed in the CRISPR-Cas9 edited lysate lane below 50 kDa is likely to represent a truncated form of Smad3. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and SMAD3 CRISPR-Cas9 edited A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-Smad3 antibody [EP568Y] (ab40854) at 1/1000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
SMAD3 CRISPR-Cas9 edited A549 cell lysate at 20 µg

Secondary

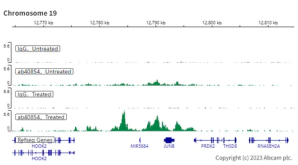
Lanes 1 - 2:
Goat anti-Rabbit IgG H&L 800CW at 1/20000 dilution

Lanes 1 - 2:
Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

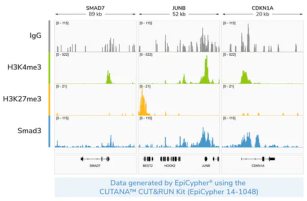
Predicted band size: 48 kDa

Observed band size: 50 kDa



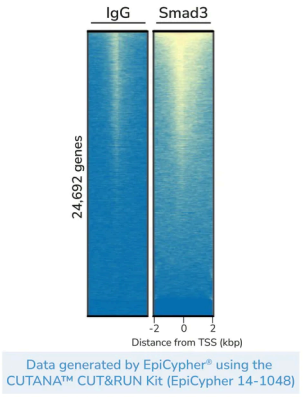
ChIC/CUT&RUN sequencing - Anti-Smad3 antibody [EP568Y] (ab40854)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10^5 A549 (Human lung carcinoma cell line) cells treated with hTGF-β1 (7 ng/mL 1 h) and 5 µg of ab40854 [EP568Y]. 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. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



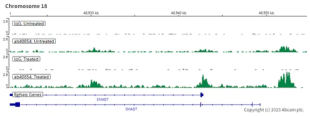
ChIC/CUT&RUN sequencing - Anti-Smad3 antibody [EP568Y] (ab40854)

CUT&RUN profiling with Smad3 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 Smad3 antibody (Abcam ab40854, 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).



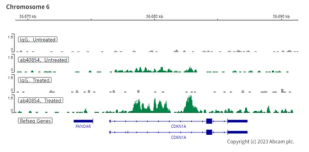
ChIC/CUT&RUN sequencing - Anti-Smad3 antibody [EP568Y] (ab40854)

CUT&RUN profiling with Smad3 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 Smad3 antibody (Abcam ab40854, 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 ChAsE (Younesy et al., *Bioinformatics* 2016; PMID 27378294). Row-linked data are ranked by intensity relative to Smad3, with red indicating high localized enrichment and blue denoting background.



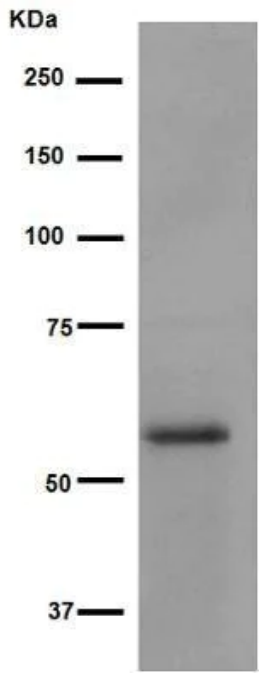
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ChIC/CUT&RUN sequencing - Anti-Smad3 antibody [EP568Y] (ab40854)

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Western blot - Anti-Smad3 antibody [EP568Y] (ab40854)

Smad3 Western blot staining of Rat liver tissue lysate using rabbit Anti-Smad3 antibody

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

All lanes:
Western blot - Anti-Smad3 antibody [EP568Y] (ab40854) at 1/5300 dilution

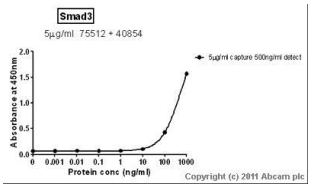
All lanes:
Rat liver tissue lysate at 10 µg

Secondary

All lanes:
Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

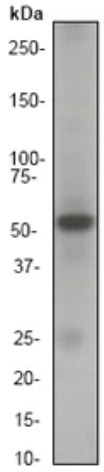
Predicted band size: 48 kDa

Observed band size: 58 kDa



Sandwich ELISA - Anti-Smad3 antibody [EP568Y] (ab40854)

Standard Curve for Smad3 (Analyte: Smad3 protein (His tag) (ab89353, unpurified)); dilution range 1pg/ml to 1µg/ml using Capture Antibody Mouse monoclonal [AF9F7] to Smad3 (ab75512) at 5µg/ml and Detector Antibody Rabbit monoclonal [EP568Y] to Smad3 (ab40854) at 0.5µg/ml.



Western blot - Anti-Smad3 antibody [EP568Y] (ab40854)

All lanes:
Western blot - Anti-Smad3 antibody [EP568Y] (ab40854) at 1/5000 dilution

All lanes:
Jurkat cell lysate at 10 µg

Predicted band size: 48 kDa

Observed band size: 55 kDa

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