Product datasheet

Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] ab52903

Overview

Product name: Anti-Smad3 (phospho S423 + S425) antibody [EP823Y]
Description: Rabbit monoclonal [EP823Y] to Smad3 (phospho S423 + S425)
Host species: Rabbit
Specificity: ab52903 detects Smad3 phosphorylated on Serine 423 and Serine 425. This Smad3 antibody may also detect Smad1, Smad2 and Smad5 phosphorylated at the equivalent sites.

Tested applications: Suitable for: WB, ICC/IF, ELISA, IHC-Fr, IHC-P, Dot blot
Unsuitable for: Flow Cyt or IP

Species reactivity: Reacts with: Mouse, Human, Drosophila melanogaster

Immunogen: A synthetic phospho specific peptide corresponding to residues surrounding Ser423 and Ser425 of human Smad3.

Positive control: WB: HL-60 treated with TGF-ß cell lysates; A549 untreated and treated with 5ng/ml TGF-ß1 for 24 hours whole cell lysates; F9 whole cell lysate. IHC-P: Human stomach and liver carcinoma tissue; Mouse kidney tissue; Environmental enteropathy (EE) duodenal biopsy. ICC/IF: TGFß treated A549 cells; PML+/+ mouse embryonic fibroblasts (MEFs) were transfected with either CTL-siRNAs or NDRG1-siRNAs; Mouse primary embryonic epicardial cells.

General notes: Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

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

This product is a recombinant rabbit monoclonal antibody.

Properties

Form: Liquid
Storage instructions: Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer: pH: 7.20
Preservative: 0.01% Sodium azide
 Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
**Purity**
Protein A purified

**Clonality**
Monoclonal

**Clone number**
EP823Y

**Isotype**
IgG

### Applications

Our **Abpromise guarantee** covers the use of **ab52903** in the following tested applications.

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>★★★★☆☆☆☆</td>
<td>1/2000. Predicted molecular weight: 48 kDa. Avoid using milk, casein, and phosphorylated proteins in general in the blocking buffer and in the antibody diluent. We recommend a solution of 5% BSA (bovine serum albumin).</td>
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<tr>
<td>ICC/IF</td>
<td>★★★★★☆☆☆☆</td>
<td>1/100 - 1/250.</td>
</tr>
<tr>
<td>ELISA</td>
<td>★★★★★☆☆☆☆</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IHC-Fr</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 23667656</td>
</tr>
<tr>
<td>IHC-P</td>
<td>★★★★★☆☆☆☆</td>
<td>1/100 - 1/250. The secondary antibody is rabbit specific IHC polymer detection kit HRP/DAB (<strong>ab209101</strong>).</td>
</tr>
<tr>
<td>IF</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 28135282</td>
</tr>
<tr>
<td>Dot blot</td>
<td></td>
<td>1/1000.</td>
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</table>

**Application notes**
Is unsuitable for Flow Cyt or IP.

### Target

**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.

**Involvement in disease**
Colorectal cancer
Loeys-Dietz syndrome 3

**Sequence similarities**
Belongs to the dwarfin/SMAD family.
Contains 1 MH1 (MAD homology 1) domain.
Contains 1 MH2 (MAD homology 2) domain.
The MH1 domain is required for DNA binding. Also binds zinc ions which are necessary for the DNA binding.
The MH2 domain is required for both homomeric and heteromeric interactions and for transcriptional regulation. Sufficient for nuclear import.
The linker region is required for the TGFbeta-mediated transcriptional activity and acts synergistically with the MH2 domain.

**Post-translational modifications**

Phosphorylated on serine and threonine residues. Enhanced phosphorylation in the linker region on Thr-179, Ser-204 and Ser-208 on EGF and TGF-beta treatment. Ser-208 is the main site of MAPK-mediated phosphorylation. CDK-mediated phosphorylation occurs in a cell-cycle dependent manner and inhibits both the transcriptional activity and antiproliferative functions of SMAD3. This phosphorylation is inhibited by flavopiridol. Maximum phosphorylation at the G(1)/S junction. Also phosphorylated on serine residues in the C-terminal SXS motif by TGFBFR1 and ACVR1. TGFBFR1-mediated phosphorylation at these C-terminal sites is required for interaction with SMAD4, nuclear location and transactivational activity, and appears to be a prerequisite for the TGF-beta mediated phosphorylation in the linker region. Dephosphorylated in the C-terminal SXS motif by PPM1A. This dephosphorylation disrupts the interaction with SMAD4, promotes nuclear export and terminates TGF-beta-mediated signaling. Phosphorylation at Ser-418 by CSNK1G2/CK1 promotes ligand-dependent ubiquitination and subsequent proteasome degradation, thus inhibiting SMAD3-mediated TGF-beta responses. Phosphorylated by PDPK1. Acetylation in the nucleus by EP300 in the MH2 domain regulates positively its transcriptional activity and is enhanced by TGF-beta.

Ubiquitinated. Monoubiquitinated, leading to prevent DNA-binding. Deubiquitination by USP15 alleviates inhibition and promotes activation of TGF-beta target genes. Poly-ADP-ribosylated by PARP1 and PARP2. ADP-ribosylation negatively regulates SMAD3 transcriptional responses during the course of TGF-beta signaling.

**Cellular localization**

TGF-β1 signaling is impaired in NDRG1-silenced MEFs. PML+/- mouse embryonic fibroblasts (MEFs) were transfected with either CTL-siRNAs (A & B) or NDRG1-siRNAs (C & D) and induced with 100 ng/ml TGF-β1. Immunofluorescent staining revealed intense nuclear staining for phosphorylated SMAD3 (SMAD3-P, ab52903) in CTL-siRNA treated MEFs (B) while only weak nuclear staining for MEFs treated with NDRG1-siRNA (D).

Representative IHC photomicrographs from an Environmental enteropathy (EE) duodenal biopsy showing p-SMAD3 staining (ab52903) in only the epithelium (arrows).
All lanes: Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903) at 1/1000 dilution (purified)

Lane 1: A549 whole cell lysate
Lane 2: A549 treated with 5ng/ml TGF-β1 for 24 hours whole cell lysate
Lane 3: A549 treated with 5ng/ml TGF-β1 for 24 hours whole cell lysate, the membrane was incubated with alkaline phosphatase

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: 48 kDa
Observed band size: 55 kDa

why is the actual band size different from the predicted?

Blocking and dilution buffer: 5% NFDM/TBST.

Purified ab52903 staining Smad3 in Human stomach tissue sections by Immunohistochemistry (Formalin/PFA fixed paraffin embedded sections). Tissue was fixed with paraffin and antigen retrieval was by heat mediation using ab93684 (Tris/EDTA buffer, Ph9.0). Samples were incubated with primary antibody at a 1/200 dilution. A ready to use rabbit specific IHC polymer detection kit HRP/DAP (ab209101). Hematoxylin was used as a counterstain. Nuclear and weakly cytoplasmic staining on human stomach without alkaline phosphatase treatment (image A). No signal can be detected when tissues were treated with alkaline phosphatase (image B).
Immunocytochemistry/Immunofluorescence analysis of A549 +/- TGFβ (5ng/ml, 24h) and A549 + TGFβ (5ng/ml, 24h) + Lambda phosphatase (LP) cells. Smad3 (phospho S423 + S425) was labelled with purified ab52903 at a dilution of 1/100 dilution, while Smad3 was labelled with ab207447 at a dilution of 1/500 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% triton X-100. ab150077 (goat anti-rabbit IgG Alexa Fluor® 488) (1/1000) was used as the secondary antibody. The cells were co-stained with ab195889 (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) 1/200. Nuclei counterstained with DAPI (blue). Control: PBS instead of the primary antibody.

Western blot - Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903) at 1/1000 dilution

**Lane 1**: HL-60 (human acute promyelocytic leukemia) treated with TGF-ß whole cell lysates, plus Smad3 non-phospho peptide

**Lane 2**: HL-60 (human acute promyelocytic leukemia) treated with TGF-ß whole cell lysates, plus Smad3 (phospho S423/425) peptide

**Lane 3**: HL-60 (human acute promyelocytic leukemia) treated with TGF-ß whole cell lysates, plus Smad2 non-phospho peptide

**Lane 4**: HL-60 (human acute promyelocytic leukemia) treated with TGF-ß whole cell lysates, plus Smad2 (phospho S465/467) peptide

**Lane 5**: HL-60 (human acute promyelocytic leukemia) treated with TGF-ß whole cell lysates, plus Smad1 non-phospho peptide

**Lane 6**: HL-60 (human acute promyelocytic leukemia) treated with TGF-ß whole cell lysates, plus Smad1 (phospho S463/465) peptide

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/2000 dilution

**Predicted band size**: 48 kDa

**Observed band size**: 55 kDa *why is the actual band size different from the predicted?*

**Exposure time**: 3 minutes

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
Purified ab52903 staining Smad3 in Mouse kidney tissue sections by Immunohistochemistry (Formalin/PFA fixed paraffin embedded sections). Tissue was fixed with paraffin and antigen retrieval was by heat mediation using ab93684 (Tris/EDTA buffer, pH 9.0). Samples were incubated with primary antibody at a 1/200 dilution. A ready to use rabbit specific IHC polymer detection kit HRP/DAP (ab209101). Hematoxylin was used as a counterstain. Nuclear and weakly cytoplasmic staining on mouse kidney without alkaline phosphatase treatment (image A). No signal can be detected when tissues were treated with alkaline phosphatase (image B).

All lanes: Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903) at 1/2000 dilution (purified)

Lane 1: F9 (Mouse embryonic testicular cancer epithelial cell) whole cell lysates
Lane 2: F9 (Mouse embryonic testicular cancer epithelial cell) whole cell lysates. Then the membrane was incubated with phosphatase.

Lysates/proteins at 15 µg per lane.

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

Predicted band size: 48 kDa
Observed band size: 50 kDa why is the actual band size different from the predicted?

Exposure time: 1 minute

Blocking and diluting buffer: 5% NFDM/TBST.
Immunohistochemical analysis of Smad3 in paraffin embedded human liver carcinoma tissue using ab52903 at 1/100 dilution.

ab52903 staining Smad3 in mouse primary embryonic epicardial cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% formaldehyde, permeabilized with 0.5% Triton X-100 and blocked with PBS + 1% BSA + 10% goat serum + 0.1% Triton X-100 for 1 hour at 20°C. Samples were incubated with primary antibody (1/100 in PBS + 1% BSA + 10% goat serum + 0.1% Triton X-100) for 16 hours at 4°C. An Alexa Fluor®488-conjugated goat anti-rabbit IgG polyclonal (1/200) was used as the secondary antibody.

**All lanes**: Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903) at 1/2000 dilution

**Lane 1**: (A) HL-60 cell lysates at 10µg untreated
**Lane 2**: (B) HL-60 cell lysates at 10µg treated with TGF.

**Predicted band size**: 48 kDa
**Observed band size**: 55 kDa
why is the actual band size different from the predicted?
**Additional bands at**: 45 kDa. We are unsure as to the identity of these extra bands.
Dot blot analysis of Smad 3 (phospho S423 + S425) phospho peptide (Lane 1), Smad 3 (phospho S423) phospho peptide (Lane 2), Smad 3 (phospho S425) phospho peptide (Lane 3) and Smad 3 non-phospho peptide (Lane 4) labelling Smad 3 (phospho S423 + S425) with ab52903 at a dilution of 1/1000. A Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) was used as the secondary antibody at a dilution of 1/20,000. Blocking and dilution buffer: 5% NFDM /TBST.

ab52903 staining Smad3 (phospho S423 + S425) in human TII Pneumocyte A549 cells by Immunocytochemistry/Immunofluorescence. Cells were fixed with paraformaldehyde and permeabilized with 0.1% Triton x100 before blocking with 3% BSA for 1 hour at RT. Samples were incubated with primary antibody (1/200: in 3% BSA in 1x PBST) for 24 hours at 4°C. A TRITC-conjugated goat polyclonal to rabbit IgG was used as secondary antibody at 1/200 dilution.

All lanes : Anti-Smad3 (phospho S423 + S425) antibody [EP823Y] (ab52903) at 1/1000 dilution

Lane 1 : Lysate prepared from untreated human A549 cells
Lane 2 : Lysate prepared from untreated human A549 cells for 30min
Lane 3 : Lysate prepared from TGF-ß1 cells at 10ng/ml for 30min
Lane 4 : Lysate prepared from TNF-a cells at 20ng/ml for 30min
Lane 5 : Lysate prepared from TGF-ß1 and TNF-a cells at above doses for 30min
Lane 6 : Blank DMEM media
Lysates/proteins at 20 µg per lane.

**Secondary**

*All lanes*: Donkey Anti-Rabbit IgG H&L (HRP) (ab16284)

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size**: 48 kDa  
**Observed band size**: 48 kDa

**Exposure time**: 1 hour

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**Please note**: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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