Monoclonal Anti-human/mouse/rat SMAD7 Antibody

ORDERING INFORMATION

Catalog Number: MAB2029

Clone: 293039

Lot Number: YHN02

Size: 100 μg

Formulation: 0.2 μm filtered solution in PBS with 5% trehalose

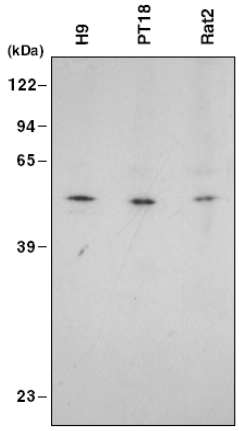
Storage: -20° C

Specificity: human/mouse/rat SMAD7

Immunogen: *E. coli*-derived recombinant human SMAD7 (aa 320 - 398)

Ig class: mouse IgG_{2B}

Recommended Applications:
Western blot
Immunohistochemistry



Detection of SMAD7 with MAB2029
Lysates from human H9, mouse PT18, and rat Rat2 cells were resolved by SDS-PAGE, transferred to Immobilon-P membrane and immunoblotted with 1.0 μg/mL mouse anti-SMAD7 as described in *Protocols for Immunoblotting*. A one minute exposure to film is shown.

Background
Smads are a family of intracellular proteins that transmit transforming growth factor beta (TGF-β) superfamily signals from the cell surface to the nucleus. The Smad family is divided into three subclasses: receptor regulated Smads, (Smads 1, 2, 3, 5 and 8); the common partner, (Smad4) that functions via its interaction to the various Smads; and the inhibitory Smads, (Smads 6 and 7). SMAD7, also known as Mothers Against Decapentaplegic homolog 7 (MADH7), inhibits selected pathways by binding directly to cell-surface receptors and preventing the activation-induced phosphorylation of other Smad subunits.

Preparation
This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, *E. coli*-derived recombinant human SMAD7 (aa 320 - 398; Accession # O15105). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

Formulation
Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution
Reconstitute with sterile PBS. If 0.2 mL of PBS is used, the antibody concentration will be 500 μg/mL.

Storage
Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity
The antibody detects endogenous human, mouse, and rat SMAD7 on Western blots.

Applications
Western blot - An antibody concentration of 1.0 μg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	2% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 1.0 μg/mL anti-human/mouse/rat SMAD7.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated goat anti-mouse IgG-HRP (R&D Systems, Catalog # HAF007).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

Cell lysates for Western blottings - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at 2 x 10^6 - 1 x 10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration.

Immunohistochemistry - This antibody was used at a concentration of 25 μg/mL with appropriate secondary reagents to detect SMAD7 in paraffin-embedded human liver cancer tissue sections. For chromogenic detection of labeling, the use of R&D Systems Cell and Tissue Staining Kits (CTS Series) is recommended.

Optimal dilutions should be determined by each laboratory for each application.

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