# *Monoclonal Anti-human Smad4 Antibody*

**ORDERING INFORMATION**

**Catalog Number:** MAB2097

**Clone:** 253343

**Lot Number:** UHK02

**Size:** 100 μg

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

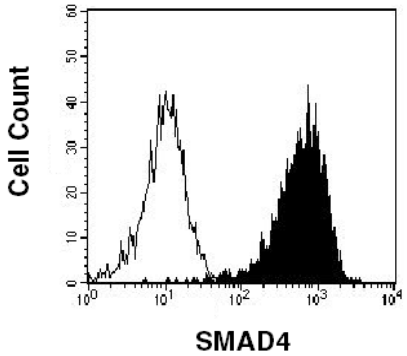
**Storage:** -20° C

**Specificity:** human Smad4

**Immunogen:** *E. coli*-derived rhSmad4

**Ig class:** mouse $IgG_{2B}$

**Recommended Applications:**
Immunocytochemistry
Flow cytometry




HeLa cells were stained with anti-Smad4 (R&D Systems, Cat. # MAB2097, filled histogram) or isotype control (R&D Systems, Cat. # MAB004, open histogram) followed by APC-conjugated anti-mouse antibody (R&D Systems, Cat. # F0101B).

## *Background*

Mothers Against Decapentaplegic homolog 4 (Smad4) belongs to a family of intracellular proteins that transmit transforming growth factor beta (TGF-β) superfamily signals from the cell surface to the nucleus. Upon signal-induced phosphorylation, Smad subunits associate with the common-mediator subunit, Smad4. This heteromeric complex then translocates into the nucleus to exert transcriptional comodulator activity.

## *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 Smad4 (rhSmad4; aa 139 - 332; Accession # Q13485). 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 recognizes rhSmad4 in direct ELISAs and Western blots.

## *Applications*

**Immunocytochemistry -** This antibody was used at a concentration of 25 μg/mL with appropriate secondary reagents to detect Smad4 in PHA-stimulated human peripheral blood mononuclear cells. For chromogenic detection of labeling, the use of R&D Systems Cell and Tissue Staining Kits (CTS Series) is recommended.

**Flow cytometry -** This antibody was tested for flow cytometry using HeLa cells. For intracellular staining to detect Smad4, cells must first be fixed and permeabilized using 4% paraformaldehyde and 0.1% saponin in PBS. Dilute this antibody to 25 μg/mL and add 10 μL of the diluted solution to 1 - 5 x $10^5$ cells in a total reaction volume not exceeding 200 μL. The binding of unlabeled monoclonal antibodies can be visualized by a secondary developing reagent such as anti-mouse IgG conjugated to a fluorochrome.

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



**R&D Systems, Inc.**
**1-800-343-7475**

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