Technical Data Sheet

PE-CF594 Mouse Anti-Smad2 (pS465/pS467)/Smad3 (pS423/pS425)

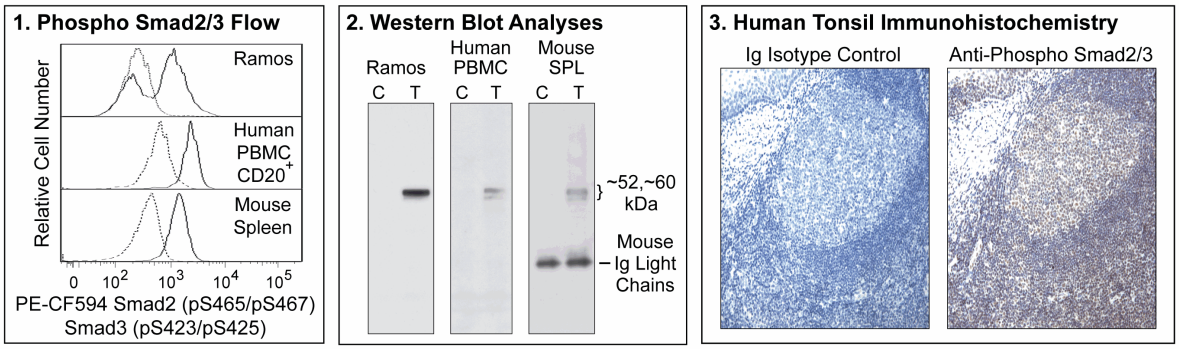
Product Information

Material Number:	**562697**
Alternate Name:	SMAD2, SMAD3; MADH2, MADH3; MAD homolog 2, MAD homolog 3
Size:	50 tests
Vol. per Test:	5 µl
Clone:	O72-670
Immunogen:	Phosphorylated Human Smad2 Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The O72-670 monoclonal antibody specifically binds to the Smad2 protein phosphorylated at the Ser465/467 sites and the Smad3 protein phosphorylated at the Ser423/425 sites. Smad2 and Smad3 are members of the Smad superfamily with observed molecular weights of 60 kDa and 52 kDa, respectively. The Smad family consists of three subfamilies: receptor regulated Smads or R-Smads, including Smads1, 2, 3, 5, and 8; common partner Smad, or Co-Smad, including Smad4; and inhibitory Smads, or I-Smad, including Smads 6 and 7. Activation of TGF-beta superfamily serine/threonine kinase receptors, such as TGF-beta, activin and BMP receptors, by their bound ligands leads to the phosphorylation of R-Smads at several sites. It has been shown that the ligand-bound TGF-beta type I receptor directly phosphorylates Ser465 and Ser467 of Smad2 and Ser423 and Ser425 of Smad3. Phosphorylated R-Smads form complexes with Co-Smad and translocate into the nucleus to regulate transcription affecting a wide range of critical cellular processes including cell-fate determination, proliferation, morphogenesis, differentiation and apoptosis. The inhibitory Smads inhibit this pathway through two potential mechanisms: either by preventing R-Smads from binding to their corresponding receptors and/or by competing with Smad4, the Co-Smad, from binding to R-Smads. High level expression of phosphorylated Smad2 has been associated with poor prognosis in late stage gastric carcinoma. Roles for Smad2 have been described in thymopoiesis and the TGF-β-mediated induction of regulatory T cells and Th17 cells. The specificity of the O72-670 mAb was confirmed by Western blot and immunohistochemistry using unconjugated antibody.

This antibody is conjugated to BD Horizon™ PE-CF594, which has been developed exclusively by BD Biosciences as a better alternative to PE-Texas Red®. PE-CF594 excites and emits at similar wavelengths to PE-Texas Red® yet exhibits improved brightness and spectral characteristics. Due to PE having maximal absorption peaks at 496 nm and 564 nm, PE-CF594 can be excited by the blue (488-nm), green (532-nm) and yellow-green (561-nm) lasers and can be detected with the same filter set as PE-Texas Red® (eg 610/20-nm filter).



Analyses of Smad2 (pS465/pS467)/Smad3 (pS423/pS425) expression.

Panel 1: *Flow cytometric analysis of Ramos cells, human PBMC, and mouse spleen cells. Cells were cultured overnight in serum-free medium (Ramos, spleen) or medium containing 0.1% FBS (PBMC) and then either not treated (dashed line histogram) or treated with TGF-β (solid line histogram; Cat. No. 354039; 10 ng/mL, 30 min, 37°C). Cells were fixed in 1X BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049; 10 min, 37°C) and permeabilized in BD Phosflow™ Perm Buffer III (Cat. No. 558050; 30 min, on ice). Cells were stained with BD Phosflow™ PE-CF594 Mouse Anti-Smad2 (pS465/pS467)/Smad3 (pS423/pS425) antibody (Cat. No. 562697). PBMC were co-stained with PerCP-Cy™5.5 Mouse Anti-Human CD20 antibody (Cat. No. 558021). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of intact cells. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.*

Panel 2: *Western blot analyses. Cells were serum-starved overnight and then either not treated (C) or treated with TGF-β (T), as in Panel 1. Lysates were blotted using Purified Mouse Anti-Smad2 (pS465/pS467)/Smad3 (pS423/pS425) antibody (Clone O72-670; 2 µg/mL). Phosphorylated Smad2 and Smad3 were identified as ~60 kDa and ~52 kDa bands, respectively. The lower molecular weight band detected in mouse spleen lysates is endogenous mouse Ig light chain.*

Panel 3: *Immunohistochemical staining. An EDTA-pretreated, formalin-fixed, paraffin-embedded human tonsil section was stained with Ig Isotype Control (Cat. No. 550878) or Purified Mouse Anti-Smad2 (pS465/pS467)/Smad3 (pS423/pS425) antibody (Clone O72-670; 20X original magnification).*

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Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with BD Horizon™ PE-CF594 under optimum conditions, and unconjugated antibody and free PE-CF594 were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

This fluorescent antibody is suitable for intracellular staining of human lymphoid cell lines, peripheral blood mononuclear cells, and mouse splenocytes using BD Cytofix™ Fixation Buffer or BD Phosflow™ Lyse/Fix Buffer with BD Phosflow™ Perm Buffer III (see table, below). Prior to stimulation, cells were serum starved overnight at a density of 2-10X10^6 cells/mL in flat-bottom 96- or 6-well tissue culture plates or in loosely capped, round-bottom tubes containing approximately 100 mL of cells in suspension.

Note:

1. Serum starvation for 2 hours following PBMC isolation was not sufficient to reduce basal phosphorylation of Smad2 and Smad3.
2. Do not mix or agitate untreated cells until just before the cells are ready to be fixed, since agitation of serum-starved mouse or human primary leukocytes prior to fixation increased Smad2/3 phosphorylation, even in the absence of exogenous TGF-β

The purified or conjugated mAb was characterized by flow cytometry (Flow), Western blot (WB), and immunohistochemistry (IHC) using these model systems:

Method	*Species*	*Cells*	*Treatment*	*Fixation*	*Perm buffer*	*Result*
Flow	Human	PBMC (serum-starved)	TGF-β	Cytofix or Lyse/Fix	Perm III	Induced, with strongest induction in CD20+ lymphocytes. S/N is higher using Lyse/Fix than using Cytofix.
	Human	Ramos	TGF-β	Cytofix	Perm III	Induced, with greatest increase over basal phosphorylation in serum-starved cells.
	Human	Jurkat (serum-starved)	TGF-β	Cytofix	Perm III	Induced
	Human	MDA-MB-231 (serum-starved)	TGF-β	Cytofix	Perm III	Induced
	Mouse	Spleen cells (serum-starved)	TGF-β + SB 431542	Lyse/Fix	Perm III	Induced by TGF-β stimulation, with induction blocked by SB 431542 ALK receptor inhibitor.
WB	Human	PBMC (serum-starved)	TGF-β			60-kDa and 52-kDa bands induced
	Human	Ramos (serum-starved)	TGF-β			60-kDa and 52-kDa bands induced
	Human	MDA-MB-231 (serum-starved)	TGF-β			60-kDa and 52-kDa bands induced.
	Mouse	Spleen cells (serum-starved)	TGF-β			60-kDa and 52-kDa bands induced
IHC	Human	Tonsil	Formalin fixed human paraffin tonsil sections with EDTA buffer pretreatment			Nuclear staining pattern

Suggested Companion Products

Catalog Number	Name	Size	Clone
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. CF™ is a trademark of Biotium, Inc.
7. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.

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9. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using multi-laser cytometers, which may directly excite both PE and CF™594.
10. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
11. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
12. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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