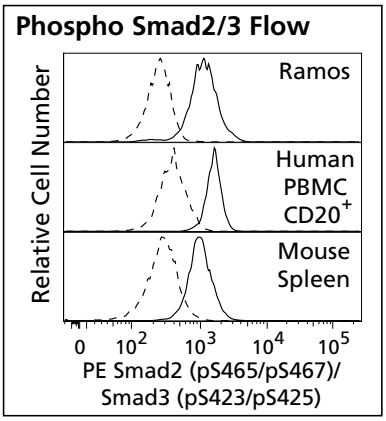
BD Phosflow™ Smad
Monoclonal Antibodies

Features

- Useful for the study of TGF-β and BMP signaling pathways
- Both antibodies are suitable for use in intracellular flow cytometry and Western blotting applications
- Tested in primary cell model systems
- Suggested assay protocols are provided

A



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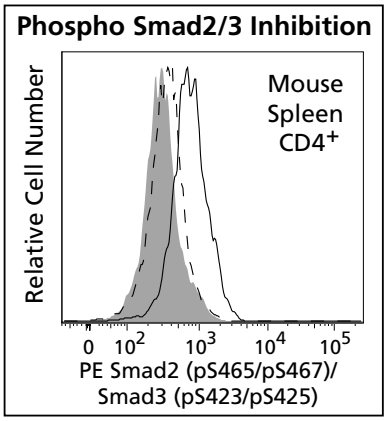

Figure 1. Analysis of Smad2 (pS465/pS467)/Smad3 (pS423/pS425) expression.
Figure A: Indicated cells were either not treated (dashed line) or treated with TGF-β (solid line). **Figure B:** Mouse splenocytes were either not treated (dashed line) or treated with TGF-β in the presence (shaded) or absence (solid line) of the SB 431542 ALK receptor inhibitor. Figure B data courtesy of Irah King and Markus Mohrs, Trudeau Institute. Please see the Technical Data Sheet for Cat. No. 562586 for experiment details.

BD Biosciences now offers purified and flourochrome-conjugated antibodies against Smad2 (pS465/pS467)/Smad3 (pS423/pS425) and Smad1 (pS463/pS465)/Smad8 (pS465/pS467) suitable for use in multiple applications.

Smad Family of Proteins
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-β superfamily serine/threonine kinase receptors (such as TGF-β, 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-β type I receptor directly phosphorylates Ser465 and Ser467 of Smad2, and Ser423 and Ser425 of Smad3.

Activation of bone morphogenic protein (BMP) receptors has been reported to phosphorylate Ser463 and Ser465 of Smad1. In B cells and pre–B cells, BMP-6 has been shown to induce Smad1/5/8 phosphorylation and inhibit cell proliferation.

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.

Anti-human Smad2 (pS465/pS467)/Smad3 (pS423/pS425) Antibody (Clone O72-670)
Clone O72-670 recognizes human and mouse Smad2 when phosphorylated on serines 465 and 467, and Smad3 when phosphorylated on serines 423 and 425, but does not recognize the unphosphorylated forms of the proteins. This antibody can detect Smad2 (pS465/pS467)/Smad3 (pS423/pS425) by flow cytometry when the cells are permeabilized with BD Phosflow™ Perm Buffer III (Figure 1). Western blot shows a single band in cells that are serum-starved and then treated with TGF-β.

Anti-human Smad1 (pS463/pS465)/Smad8 (pS465/pS467) Antibody (Clone N6-1233)
Smad1 and Smad8 are recognized by clone N6-1233 when phosphorylated on pS463/pS465 and pS465/pS467, respectively. This antibody does not recognize the non-phosphorylated form of the protein. This antibody is useful for intracellular flow cytometry when used with BD Phosflow Perm Buffer III. By Western blot, this antibody detects a single band of approximately 60 kDa (Figure 2).

Visit **bdbiosciences.com** for more information.

BD Phosflow™ Smad Monoclonal Antibodies

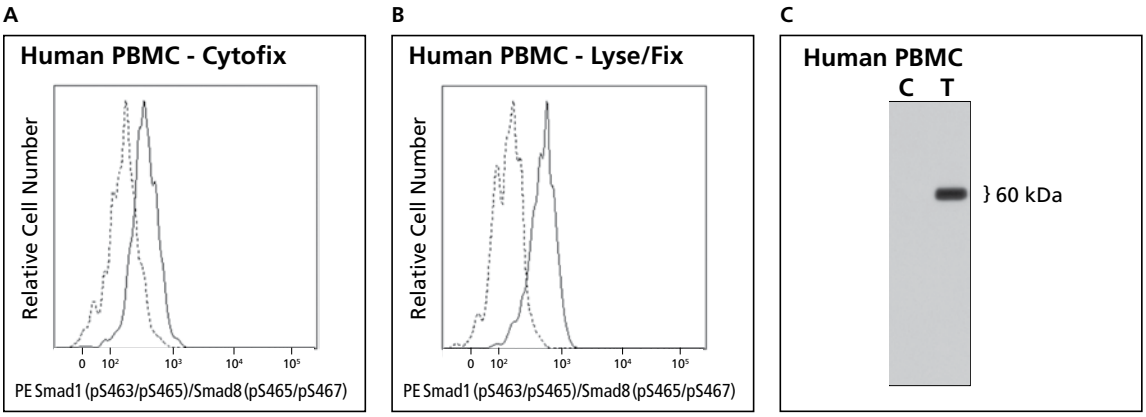

Figure 2. Analyses of Smad1 (pS463/pS465)/Smad8 (pS465/pS467) expression by human peripheral blood mononuclear cells (PBMCs).

Figures A and B: PBMCs were either not treated (dashed line) or treated with BMP-6 (solid line). Cells were fixed in BD Cytofix™ fixation buffer (Figure A) or 1X BD Phosflow™ Lyse/Fix buffer (Figure B), and then permeabilized with BD Phosflow Perm Buffer III. **Figure C:** Western blot. Lysates were prepared from PBMCs that were either not treated (C) or treated (T) with BMP-6. Detects a band of ~60 kDa. Please see the Technical Data Sheet for Cat. No. 562508 and 562509 for experiment details.

Table 1. The purified or conjugated N6-1233mAb was characterized by flow cytometry (Flow), Western blot (WB), and immunohistochemistry (IHC) using these model systems:

	Species	Cells	Treatment	Fixation	Perm Buffer	Result
Flow	Human	PBMC (serum-starved)	BMP-6	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 (serum-starved)	BMP-6	Cytofix	Perm III	Induced
	Human	SHSY5Y (serum-starved)	BMP-2 + peptide blocking	Cytofix	Perm III	Induced. Blocked by pS463/pS465 phospho peptide but not by non-phospho peptide.
WB	Human	PBMC (serum-starved)	BMP-6			60-kDa band induced
	Human	Ramos (serum-starved)	BMP-6			60-kDa band induced
	Human	SHSY5Y (serum-starved)	BMP-2 + peptide blocking			60-kDa band induced. Blocked by pS463/pS465 phospho peptide but not by non-phospho peptide.
IHC	Human	Breast and lung cancer	Paraffin sections of human breast cancer and lung cancer with EDTA buffer pretreatment			No staining observed

Ordering Information

Description	React.	Clone	Isotype	Format	Size	Cat.No.
Smad1 (pS463/pS465)/Smad8 (pS465/pS467)	Hu	N6-1233	Rat IgG_{2a}, κ	Purified	0.1 mg	**562508**
				PE	50 tests	**562509**
Smad2 (pS465/pS467)/Smad3 (pS423/pS425)	Hu	O72-670	Ms IgG_1, κ	PE	50 tests	**562586**

Related Products

Description	React.	Clone	Isotype	Format	Size	Cat.No.
BD Cytofix fixation buffer	–	–	–	Buffer	100 mL	**554655**
BD Phosflow Lyse/Fix buffer	–	–	–	Buffer	250 mL	**558049**
BD Phosflow Perm Buffer III	–	–	–	Buffer	125 mL	**558050**

References

1. Abdollah S, Macías-Silva M, Tsukazaki T, Hayashi H, Attisano L, Wrana JL. TbetaRI phosphorylation of Smad2 on Ser465 and Ser467 is required for Smad2-Smad4 complex formation and signaling. *J Biol Chem*. 1997;272:27678-27685.
2. Matsuzaki K. Smad phosphoisoform signaling specificity: the right place at the right time. *Carcinogenesis*. 2011;32:1578-1588.
3. Kretzschmar M, Doody J, Massagué J.Opposing BMP and EGF signalling pathways converge on the TGF-beta family mediator Smad1. *Nature*. 1997;389:618-622.

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