Technical Data Sheet

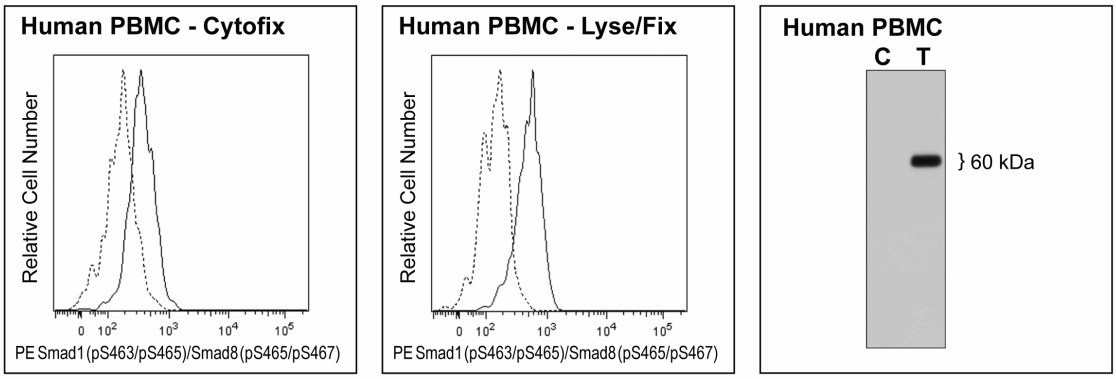
PE Rat anti-Smad1 (pS463/pS465)/Smad8 (pS465/pS467)

Product Information

Material Number:	**562509**
Alternate Name:	Smad1,Smad8/Smad9; MADH1, MADH9
Size:	50 tests
Vol. per Test:	5 µl
Clone:	N6-1233
Immunogen:	Phosphorylated Human Smad1 aa 456-465 Peptide
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Human. Predicted due to immunogen sequence identity: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The N6-1233 monoclonal antibody specifically binds to the Smad1 protein phosphorylated at the Ser463/465 sites and the Smad8 protein phosphorylated at the Ser465/467 sites. Smad1 and Smad8 are ~60 kDa proteins and are members of the Smad superfamily. The Smad family members are divided into 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 Transforming Growth Factor-beta (TGF-beta) superfamily serine/threonine kinase receptors, such as TGF-beta and Bone Morphogenic Protein (BMP) receptors, leads to the phosphorylation of R-Smads at several sites. It has been shown that Ser463 and Ser465 of Smad1 are phosphorylated by BMP receptors. 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 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. This antibody may crossreact with Smad5 pS463/pS465 based on sequence homology.



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

Multicolor flow cytometric analysis of Smad1 (pS463/pS465)/Smad8 (pS465/pS467) expressed by PBMC. *Cells were cultured overnight in media containing 0.1% FBS and were then either not treated (dashed line histogram) or treated with Bone Morphogenetic Protein 6 (BMP-6; R&D Systems, Cat. No. 507-BP; 500 ng/ml, 15 min, 37°C; solid line histogram). Cells were fixed in BD Cytofix™ Fixation Buffer (Cat. No. 554655; 10 min, 37°C; Left Panel) or 1X BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049; 10 min, 37°C; Middle Panel) and permeabilized in BD Phosflow™ Perm Buffer III (Cat. No. 558050; 30 min, on ice). Cells were then stained with BD Phosflow™ PE Rat anti-Smad1 (pS463/pS465)/Smad8 (pS465/pS467) (Cat. No. 562509) and Alexa Fluor® 647 Mouse Anti-Human CD20 (Cat. No. 558054) mAbs. Fluorescence histograms showing Smad1 (pS463/pS465)/Smad8 (pS465/pS467) expression were generated for CD20-positive gated events with the forward and side-light scatter characteristics of intact cells using a BD FACSCanto™ II Flow Cytometer System.*

Western blot analysis of Smad1 (pS463/pS465)/Smad8 (pS465/pS467) expression by human PBMC. *Lysates were prepared from 1 million PBMC that were cultured overnight in media containing 0.1% FBS and were then either not treated (C) or treated (T) with BMP-6 (500 ng/ml, 15 min, 37°C). The lysates were electrophoresed, transferred to a membrane and blotted using Purified Rat anti-Smad1 (pS463/pS465)/Smad8 (pS465/pS467) mAb (Cat. No. 562508; 0.125 µg/ml for Lanes 1 and 2), HRP Goat Anti-Rat Ig (Cat. No. 554017) and a chemiluminescent detection system. Smad1 (pS463/pS465)/Smad8 (pS465/pS467) were identified as ~60 kDa bands by Westren blotting (Right Panel).*

Note: *For all analyses shown, PBMC were isolated from freshly drawn EDTA blood.*

Preparation and Storage

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

The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

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

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

In human PBMC, overnight serum starvation was found to be necessary for detection of a BMP-6-induced increase in Smad1/8 phosphorylation. Serum starvation for 1 hour following PBMC isolation was not sufficient to reduce basal levels of Smad1 (pS463/pS465)/Smad8(pS465/pS467). Some donor variation was observed in the reduction of basal phosphorylation by overnight serum starvation.

The purified or conjugated mAb 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			No staining observed

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
554655	Fixation Buffer	100 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
558054	Alexa Fluor® 647 Mouse Anti-Human CD20	50 tests	H1
562508	Purified Rat anti-Smad1 (pS463/pS465)/Smad8 (pS465/pS467)	0.1 mg	N6-1233

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 refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

References

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