BD Phosflow™

Technical Data Sheet

Alexa Fluor® 647 Anti-Smad2 (pS465/pS467)/Smad3 (pS423/pS425)

Product Information

Material Number: 562696
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-Smads, 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.

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 to Alexa Fluor® 647 under optimum conditions, and unreacated Alexa Fluor® 647 was removed.

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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 2-10×10⁶ cells 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:

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

Suggested Companion Products

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>558049</td>
<td>Lyse/Fix Buffer 5X</td>
<td>250 ml</td>
<td>(none)</td>
</tr>
<tr>
<td>558050</td>
<td>Perm Buffer III</td>
<td>125 ml</td>
<td>(none)</td>
</tr>
</tbody>
</table>

Product Notices
1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10⁶ cells in a 100-µl experimental sample (a test).
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
7. 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.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
9. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.

References

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