

**DESCRIPTION**

<b>Species Reactivity</b>	<i>Drosophila</i>
<b>Specificity</b>	Detects <i>Drosophila</i> Smad2 in direct ELISAs and Western blots. In direct ELISAs, approximately 10% cross-reactivity with recombinant human (rh) Smad1 is observed, and less than 1% cross-reactivity with rhSmad2, rhSmad3, and rhSmad5 is observed.
<b>Source</b>	Polyclonal Sheep IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	<i>E. coli</i> -derived recombinant <i>Drosophila</i> Smad2 Gly262-Ser486 Accession # O96660
<b>Formulation</b>	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

**APPLICATIONS**

**Please Note:** Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	<b>Recommended Concentration</b>	<b>Sample</b>
<b>Western Blot</b>	0.1-0.7 µg/mL	See Below
<b>Immunohistochemistry</b>	0.1-0.7 µg/mL	See Below

**DATA**

**Western Blot**

**Detection of *Drosophila* Smad2 by Western Blot.** Western blot shows recombinant *Drosophila* Smad2 (2 ng/lane). PVDF membrane was probed with 0.1 µg/mL of Sheep Anti-*Drosophila* Smad2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7948) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Smad2 (C-terminal fragment) at approximately 25 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

**Western Blot**

**Detection of *Drosophila* Smad2 by Western Blot.** Western blot shows wild-type and Smad2 null mutation *Drosophila* larval extracts. PVDF membrane was probed with 0.7 µg/mL of Sheep Anti-*Drosophila* Smad2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7948) followed by Anti-Sheep IgG Secondary Antibody. A specific band was detected for Smad2 at the expected mobility of approximately 58 kDa, but not mutant extracts. This experiment was conducted under reducing conditions. dSmad2 null mutation is described in Peterson, AJ, *et al.* (2012) PLoS One 7: e36548. Image courtesy of Dr. Aidan Peterson and Dr. Michael O'Connor, Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, Minnesota, USA.

**Immunohistochemistry**

**Smad2 in *Drosophila* Larvae.** Smad2 was detected in wild-type and Smad2 null mutation *Drosophila* larvae using Sheep Anti-*Drosophila* Smad2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7948) at 0.7 µg/mL. Tissue was stained using a fluorescent Anti-Sheep IgG Secondary Antibody. A single confocal image in the upper panel depicts detection of endogenous Smad2 in epidermal and central nervous system cells. The dSmad2 mutant embryo in the lower panel was stained and imaged identically and reveals background staining. dSmad2 null mutation is described in Peterson, AJ, *et al.* (2012) PLoS One 7: e36548. Image courtesy of Dr. Aidan Peterson and Dr. Michael O'Connor, Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, Minnesota, USA.

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Sterile PBS to a final concentration of 0.2 mg/mL.
<b>Shipping</b>	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>• 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

**BACKGROUND**

Smad2 (SMALL body plus Mothers Against Decapentaplegic; also SmoX [Smad on Chr X]) is a 54 kDa (predicted) member of the receptor regulated Smad family of proteins. It is a downstream component of the *Drosophila* activin/TGF $\beta$  signaling pathway, and appears to play a key role in cell proliferation. Analogous to the system in vertebrates, stimulation of the fruitfly activin type I receptor (baboon):type II receptor (punt) complex results in dSmad2 (*Drosophila* Smad2) phosphorylation and subsequent interaction with dSmad4/Medea. As in vertebrates, the *Drosophila* Smad2/Smad4 complex enters the nucleus and interacts with a transcriptional cofactor (s) termed TGIF. At this point, the vertebrate:invertebrate systems diverge over the nature of the TGIF cofactor(s); vertebrate TGIF is a corepressor, while invertebrate TGIF (dTGIF) is a coactivator. *Drosophila* Smad2 is 486 amino acids (aa) in length. It contains two Mad homology domains (aa 7-130 and 285-475), the former of which participates in DNA-binding. Over aa 262-486, *Drosophila* Smad2 shares 70% aa sequence identity with both mouse and human Smad2.