

**DESCRIPTION**

<b>Species Reactivity</b>	Mouse/Rat
<b>Specificity</b>	Detects mouse and rat SP-D in Western blots. In direct ELISAs, approximately 20% cross-reactivity with recombinant human SP-D is observed.
<b>Source</b>	Polyclonal Sheep IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	Chinese hamster ovary cell line CHO-derived recombinant mouse SP-D Ala20-Phe374 Accession # P50404
<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 either lyophilized or 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>	2 µg/mL	See Below
<b>Simple Western</b>	20 µg/mL	See Below


**DATA**

**Western Blot**

**Detection of Mouse and Rat SP-D by Western Blot.** Western blot shows lysates of mouse lung tissue and rat lung tissue. PVDF membrane was probed with 2 µg/mL of Sheep Anti-Mouse/Rat SP-D Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6839) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for SP-D at approximately 43 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

**Simple Western**

**Detection of Rat and Mouse SP-D by Simple Western™.** Simple Western lane view shows lysates of rat lung tissue and mouse lung tissue, loaded at 0.2 mg/mL. A specific band was detected for SP-D at approximately 60 kDa (as indicated) using 20 µg/mL of Sheep Anti-Mouse/Rat SP-D Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6839) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



**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**

SP-D (surfactant protein-D; also PSP-D) is a 43 kDa member of the collectin family of innate immune modulators (1 - 5). It is constitutively secreted by alveolar lining cells and epithelium associated with tubular structures. SP-D is found in serum, plasma, broncho-alveolar lavage (BAL) fluid, and amniotic fluid (1, 2, 6). Lung injuries often increase release of SP-D to the circulation (3, 6). Mouse SP-D is synthesized as a 374 amino acid (aa) precursor. Mouse SP-D cDNA encodes a 19 aa signal sequence and a 355 aa mature region with a 25 aa N-terminal linking-region, a 177 aa hydroxyproline and hydroxylysine collagen-like domain, a 46 aa coiled-coil segment, and a 106 aa, C-terminal collectin-like C-type lectin domain (CRD) (5). Mature mouse SP-D shares 72 - 76% aa sequence identity with human, porcine, equine, canine and bovine SP-D, and 92% with rat SP-D. SP-D is usually found as a glycosylated, disulfide-linked 150 kDa  $\alpha$ -helical coiled-coil trimer with a "head" of three symmetrical CRDs (2-4, 7). Each CRD recognizes the hydroxides of one monosaccharide, and trimerization allows for the discrimination of monosaccharide patterns specific to microbial pathogens (4, 7, 8). Typically, SP-D forms a higher-order 620 kDa, X-shaped dodecamer through N-terminal disulfide bonds, allowing for even finer discrimination of self vs. nonself carbohydrate patterns and facilitating binding to complex antigens (1). SP-D also binds SIRP $\alpha$  and the calreticulin/CD91 complex on macrophages (9, 10). When the ratio of antigen/pathogen to available CRDs is low, antigen can be bound without occupying all available CRDs. The free CRDs will bind to SIRP $\alpha$ , generating a signal that downmodulates the inflammatory response. During high CRD ligand binding (low SIRP $\alpha$  binding), the dodecamer rearranges to expose N-termini that bind the calreticulin/CD91 complex, an event that initiates inflammation (1). Also, direct and indirect binding of neutrophil defensins and macrophage CD14 and TLRs to SP-D can modulate response to viruses and bacterial lipopolysaccharides (1-3, 11-15). Thus, SP-D allows for a graded response to environmental challenge and clearance of small antigenic insults without the need for a damaging inflammatory response (1-3).

**References:**

1. Forbes, L.R. and A. Haczk (2010) Clin. Exp. Allergy **40**:547.
2. Kishore, U. *et al.* (2006) Mol. Immunol. **43**:1293.
3. Hartl, D. and M. Griese (2006) Eur. J. Clin. Invest. **36**:423.
4. Sim, R.B. *et al.* (2006) Novartis Found Symp. **279**:170.
5. Motwani, M. *et al.* (1995) J. Immunol. **155**:5671.
6. Honda, Y. *et al.* (1995) Am. J. Respir. Crit. Care Med. **152**:1860.
7. Hakansson, K. *et al.* (1999) Structure **7**:225.
8. Crouch, E.C. *et al.* (2006) Am. J. Respir. Cell Mol. Biol. **35**:84.
9. Janssen, W.J. *et al.* (2008) Am. J. Respir. Crit. Care Med. **178**:158.
10. Gardai, S.J. *et al.* (2003) Cell **115**:13. Ohya, M. *et al.* (2006) Biochemistry **45**:8657.
11. Ohya, M. *et al.* (2006) Biochemistry **45**:8657.
12. Pastva, A.M. *et al.* (2007) Proc. Am. Thorac. Soc. **4**:252.
13. Sano, H. and Y. Kuroki (2005) Mol. Immunol. **42**:279.
14. Hartshorn, K.L. *et al.* (2006) J. Immunol. **176**:6962.
15. Yamazoe, M. *et al.* (2008) J. Biol. Chem. **283**:35878.