

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human SP-D in direct ELISAs and human, mouse, and rat SP-D in Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human SP-D Ala21-Phe375 (Glu22Gly) Accession # P35247
Formulation	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.

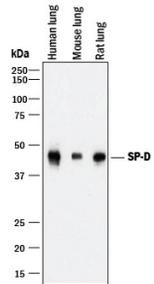
APPLICATIONS

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.

	Recommended Concentration	Sample
Western Blot	0.2 µg/mL	See Below
Simple Western	10 µg/mL	See Below

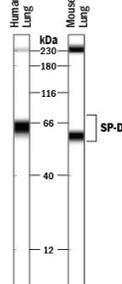
DATA

Western Blot



Detection of Human, Mouse, and Rat SP-D by Western Blot. Western blot shows lysates of human lung tissue, mouse lung tissue, and rat lung tissue. PVDF membrane was probed with 0.2 µg/mL of Goat Anti-Human/Mouse/Rat SP-D Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1920) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). 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 Human and Mouse SP-D by Simple Western™. Simple Western lane view shows lysates of human lung tissue and mouse lung tissue, loaded at 0.2 mg/mL. A specific band was detected for SP-D at approximately 64/59 kDa (as indicated) using 10 µg/mL of Goat Anti-Human/Mouse/Rat SP-D Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1920) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system. Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody.



PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	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
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

SP-D (surfactant protein-D; also PSP-D) is a 43 kDa member of the collectin family of innate immune modulators. It is constitutively secreted by alveolar lining cells and epithelium associated with tubular structures. Its principal components consist of a collagen-like region and a C-terminal carbohydrate recognition domain (CRD), a structure that further places it in a subset of an expanded group of proteins termed defense collagens (1-4). Human SP-D is synthesized as a 375 amino acid (aa) precursor. It contains a 20 aa signal sequence and a 355 aa mature region. The mature molecule is characterized by the presence of 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, 6). Two additional, potential isoforms exist. One shows a 13 aa N-terminal extension, while the other combines the N-terminal extension with a deletion of aa's 206-375. Mature human SP-D shares 75% and 78% aa identity with mouse and pig SP-D, respectively. Monomeric SP-D is unusual (3). The basic form of SP-D is that of a glycosylated, disulfide-linked 150 kDa trimer that generates an α -helical coiled-coil structure linked to a "head" of three symmetrical CRDs (4, 7). Each CRD recognizes the hydroxides of one monosaccharide (4). Trimerization allows for the discrimination of monosaccharide patterns specific to microbial pathogens (7). Typically, SP-D forms a higher-order 620 kDa, X-shaped dodecamer through disulfide bonds associated with the N-terminus (8). This allows for even finer discrimination of self vs. nonself carbohydrate patterns, and facilitates binding to complex antigens (8, 9). One polymorphism, a Met11-Thr11 transition in human, apparently precludes the formation of oligomers, potentially affecting the ability of affected individuals to interact with microorganisms (9, 10). Finally, SP-D is known to bind both SIRP α and the calreticulin/CD91 complex on macrophages. 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 α , generating a signal that downmodulates the inflammatory response. When virtually all CRDs are occupied by ligand, however, free CRDs are not available for SIRP α binding. Instead, the dodecamer is depicted to undergo a structural rearrangement, exposing the N-termini of all four linked trimers. This exposed terminus is known to bind to the calreticulin/CD91 complex, an event that initiates inflammation. Thus, it would appear that SP-D allows for a graded response to environmental challenge. SP-D provides a mechanism for the clearance of small antigenic insults without the need for a damaging inflammatory response (3).

References:

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