

# **Bovine SPAM1 Antibody**

Antigen Affinity-purified Polyclonal Sheep IgG Catalog Number: AF6436

DESCRIPTION			
Species Reactivity	Bovine		
Specificity	Detects bovine SPAM1 in direct ELISAs and Western blots.		
Source	Polyclonal Sheep IgG		
Purification	Antigen Affinity-purified		
Immunogen	Chinese hamster ovary cell line CHO-derived recombinant bovine SPAM1 Leu36-Thr497 Accession # AAI10184		
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 either lyophilized or as a 0.2 µm filtered solution in PBS.		

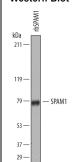
#### APPLICATIONS

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.

	Recommended Concentration	Sample
Western Blot	1 μg/mL	See Below

#### DATA

### Western Blot



Detection of Bovine SPAM1 by Western Blot. Western blot shows lysates of recombinant bovine SPAM1 (Catalog # 6436-GH). PVDF membrane was probed with 1 µg/mL of Sheep Anti-Bovine SPAM1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6436) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for SPAM1 at approximately 78 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.

# PREPARATION AND STORAGE

**Reconstitution** Sterile PBS to a final concentration of 0.2 mg/mL.

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.

- 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.

#### BACKGROUNI

Sperm adhesion molecule 1 (SPAM1), also known as PH-20 hyaluronidase (1), is encoded by one of the six hyaluronidase-like genes (1, 2, 3). SPAM1 is a GPI-anchored enzyme located on the sperm surface and inner acrosomal membrane (4). SPAM1 degrades hyaluronic acid (HA), a major structural glycosaminoglycan found in extracellular matrices and basement membranes. The enzyme activity enables sperm to penetrate through the HA-rich cumulus cell layer surrounding the oocyte and therefore facilitates the fertilization process (5). However, detailed enzymatic analysis of this enzyme is hindered by the limited techniques/methods available to monitor the HA degradation products (6). A novel method for analyzing SPAM1 activity was utilized here. Because of the structural similarity between HA (repeating units of GlcAβ1-3GlcNAc) and chondroitin sulfate (repeating units of GlcAβ1-3GalNAc), the enzyme is also able to hydrolyze chondroitin sulfate. In this assay, radiolabeled chondroitin sulfate was digested with recombinant SPAM1. Degradation products were then separated using a polyacrylamide electrophoresis and visualized with an X-ray film (7). The bovine SPAM1 is 63% identical to human homologue in sequence.

# References:

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- 6. Hofinger, E.S et al. (2007) Glycobiology 17:963.
- 7. Wu, Z.L. et al. (2010) BMC Biotechnol. 10:11. 8.
- 8. Robbins, P.W. (1962) Methods in Enzymology, Vol. V, Academic Press, Inc., New York, 964.
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