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### HRP-labeled Goat Anti-SAP GOAT POLYCLONAL

**Catalog Number:** AB-15HRP  
**Quantity:** 200 micrograms  
**Format:** PBS (0.14 M Sodium Chloride; 0.003 M Potassium Chloride; 0.002 M Potassium Phosphate; 0.01 M Sodium Phosphate; pH 7.4), no preservative.  
**Host:** Goat  
**Isotype:** IgG  
**Immunogen:** Saporin

#### **Background:**

Saporin is obtained from the seeds of the Soapwort plant (*Saponaria officinalis*), a plant that grows wild in Britain and other parts of Europe. Saporin is a plant enzyme with N-glycosidase activity that depurinates a specific nucleotide in the ribosomal RNA 28S, thus irreversibly blocking protein synthesis. It belongs to the well-characterized family of ribosome-inactivating proteins (RIPs). There are two types of RIPs: type I, which are much less cytotoxic due to the lack of the B chain and type II, which are distinguished from type I RIPs by the presence of the B chain and their ability to enter cells on their own. However, type I RIPs can still be internalized by fluid-phase endocytosis. In the case of saporin, it was reported that saporin first binds to the alpha2-macroglobulin receptor on human cells and is then internalized to the cytosol. Upon internalization, the ribosomes are inactivated, resulting in cell death.

HRP-labeled Anti-SAP can be used to verify binding specificity of a targeted toxin to a cell line expressing the target molecule. By first binding the targeted toxin to protein extract or plate-bound antigen, then binding HRP-labeled Anti-SAP to the targeted toxin, specificity can be confirmed through the use of competing molecules or a control cell line.

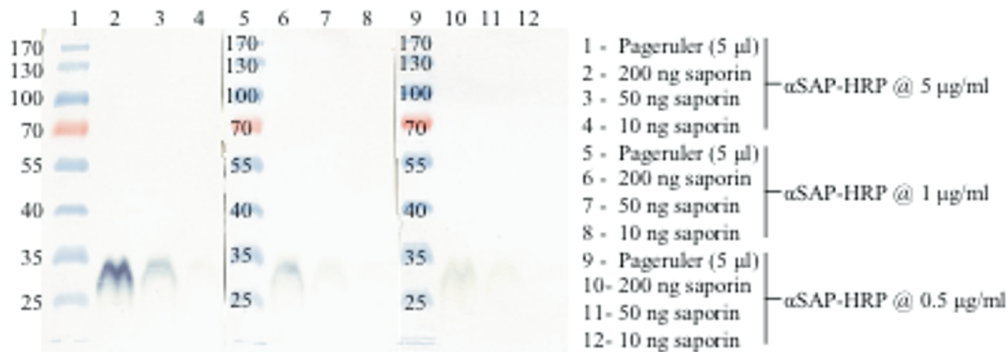
#### **Specificity and Preparation:**

This antibody recognizes saporin. Saporin was used as the immunogen. The antibody was coupled to Horseradish Peroxidase (HRP) and dialyzed against PBS. The conjugated antibody is routinely tested by western blot.

#### **Usage and Storage:**

Applications include immunohistochemistry (frozen, fixed sections; 1:1000 or 1:350)<sup>1,2</sup> and immunoblotting (0.5  $\mu$ g/ml-5  $\mu$ g/ml, ATS in-house). Store the material at 4°C for one month or -20°C in undiluted aliquots for up to one year. Avoid repeated freezing and thawing. Gently spin down material before use; 5-10 seconds in a microfuge should be adequate.

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Saporin (200, 50, and 10 ng) was run on a 10% SDS-PAGE gel and transferred to PVDF membrane. The blot was blocked with 4% NFM/TBS, then incubated overnight with 0.5 µg/ml (Lanes 9-12), 1 µg/ml (Lanes 5-8), or 5 µg/ml (Lanes 1-4) of antibody. The blot was washed and developed with 4-chloro-1-naphthol and hydrogen peroxide.

**References:**

1. Vulchanova L, Olson TH, Stone LS, Riedl MS, Elde R, Honda CN (2001) Cytotoxic targeting of isolectin IB4-binding sensory neurons. *Neurosci* 108(1):143-155.
2. Mantyh PW, Rogers SD, Honore P, Allen BJ, Ghilardi JR, Li J, Daughters RS, Lappi DA, Wiley RG, Simone DA (1997) Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor. *Science* 278:275-279.

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