ADVANCED TARGETING SYSTEMS

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Saporin

a ribosome-inactivating protein from *Saponaria officinalis*

Catalog Number: PR-01
Quantity: 100 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. Sterile-filtered.

Background:
Saporin is obtained from the seeds of the Soapwort plant (*Saponaria officinalis*), a plant that grows wildly 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.

Specificity and Preparation:
Saporin (molecular weight 30 kDa) has no known specificity. Saporin is purified from the seeds of the Soapwort plant (*Saponaria officinalis*). The product is routinely tested for activity by a protein synthesis inhibition assay.

Usage and Storage:
Saporin serves as a control for targeted saporin immunotoxins or ligand toxins.
Gently spin down material before use; 5-10 seconds in a microfuge should be adequate. Aliquot and store frozen at -20 or -80°C. Avoid repeated freezing and thawing. For disposal: autoclave, or expose to 0.2 M NaOH, materials that come into contact with the toxin.
Sample Protocol: For most targeted toxins, there are control conjugates available; these are the most accurate controls to use. For a few products, saporin is used as control. Calculate amount of saporin needed on a molar basis. For example, FGF-SAP (Cat. #IT-38) has an average molecular weight of 63 kDa, and 71% of that is saporin (approximately 1.5 molecules of saporin per molecule of cytokine). For injections of 5 μg of immunotoxin, an appropriate control quantity would be 3.6 μg.

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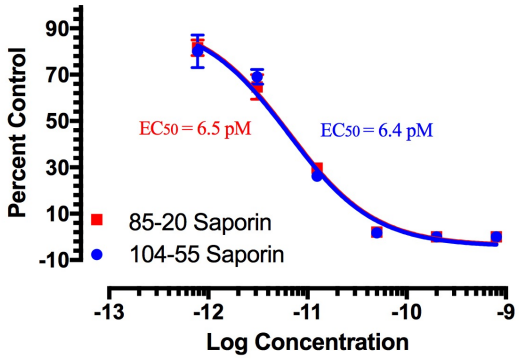
References:

1. Stirpe F, Barbieri L, Battelli MG, Soria M, Lappi DA. (1992) Ribosome-inactivating proteins from plants: present status and future prospects. *Bio/Technol* 10:405-412.
2. Lappi DA, Esch FS, Barbieri L, Stirpe F, Soria M. (1985) Characterization of a *Saponaria officinalis* seed ribosome-inactivating protein: immunoreactivity and sequence homologies. *Biochem Biophys Res Commun* 129:934-942.
3. Stirpe F, Gasperi-Campani A, Barbieri L, Falasca A, Abbondanza A, Stevens WA. (1983) Ribosome-inactivating proteins from the seeds of *Saponaria officinalis* L. (soapwort) of *Agrostemma githago* L. (corn cockle) and of *Asparagus officinalis* (asparagus) and from the latex of *Hura crepitans* L. (sandbox tree). *Biochem J* 216:617-625.

To view protocol(s) for this and other products please visit: www.ATSbio.com/support/protocols



Saporin activity is tested by protein synthesis inhibition assay. RNAase-free 0.7 ml microcentrifuge tubes are labeled and put on ice. 7 µl of rabbit reticulocyte lysate (Promega) and 2 µl of amino acid/luciferase mRNA (Promega) reagent mixture are added to each tube. Saporin dilutions are made into water and 1 µl of each saporin dilution sample is added to a corresponding tube. Tubes are incubated for 30 minutes in a 30°C water bath. 90 µl of water are added to each tube. Samples are read on a Lumat LB 9501 luminometer using Luciferase Assay Reagent (Promega). Data is analyzed using Prism software (Graphpad). Note: Lot #117-65 is a dilution of Lot #104-55.