ADVANCED TARGETING SYSTEMS

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Bombesin-SAP

TARGETED SAP CONJUGATE

a tool for eliminating cells that express gastrin releasing peptide (GRP) receptor; targeted via bombesin, eliminated via saporin

Catalog Number: IT-40
Quantity: 25 micrograms, 100 micrograms, 250 micrograms, 1 milligram
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:
Targeted SAP conjugates are powerful and specific lesioning agents used in the technique known as Molecular Surgery. The ribosome-inactivating protein, saporin (from the seeds of the plant, *Saponaria officinalis*) is bound to a targeting agent (anything that is recognized on the cell surface and internalized). The targeted conjugate is administered to cells (*in vitro* or *in vivo*). The targeting agent seeks out and binds to its target on the cell surface. The conjugate is internalized, saporin breaks away from the targeting agent, and inactivates the ribosomes which causes protein inhibition and, ultimately, cell death. Cells that do not have the cell surface marker are not affected.
Bombesin is a 14-amino-acid peptide found in frog-skin. The human equivalent, gastrin releasing peptide (GRP) has been detected in itch pathways and plays a role in eating behaviors. GRP regulates numerous functions of the gastrointestinal and central nervous systems, including release of gastrointestinal hormones, smooth muscle cell contraction, and epithelial cell proliferation. Elimination of cells expressing GRP receptor is useful in studying the role of bombesin in itch and eating behaviors.

Specificity and Preparation:
This targeted toxin (molecular weight 31.8 kDa) targets GRP receptor-positive cells. Bombesin-SAP is a chemical conjugate of bombesin and the ribosome-inactivating protein, saporin (Cat. #PR-01).

Usage and Storage:
Bombesin-SAP specifically targets GRP receptor-positive cells. **There may be lot-to-lot variation in material; working dilutions must be determined by end user. If this is a new lot, you must assess the proper working dilution before beginning a full experimental protocol.**
Gently spin down material before use; 5-10 seconds in a microfuge should be adequate. Store the material in undiluted aliquots at –20°C for 1-2 months. For longer term storage store the material at –80°C. Material should be aliquoted to a convenient volume and quantity to avoid repeated freezing and thawing that can damage the protein content. Under these conditions, the material has a very stable shelf-life. Thawing should be done at room temperature or on ice. The thawed solution should remain on ice until use.
Do not use a reducing agent (such as dithiothreitol, beta-mercaptoethanol or ascorbic acid) with this material. It will inactivate the toxin.
This material is an extremely potent cytotoxin. Handling should be done by experienced personnel. Gloves and safety glasses are required when handling this product. Care in disposal is mandatory; autoclaving or exposure to 0.2 M sodium hydroxide will inactivate the material. All labware that comes into contact with this material should be likewise treated.

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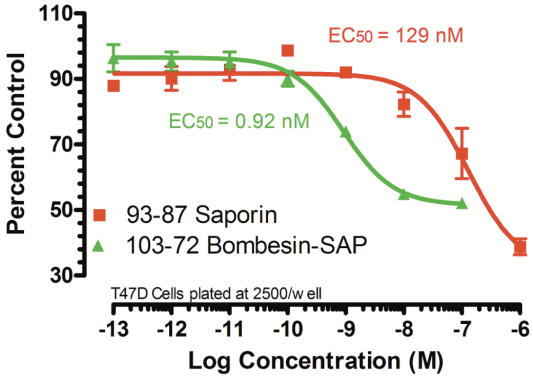
Available Control(s): Blank-SAP

Safety:
Good laboratory technique must be employed for safe handling of this product.
This requires observation of the following practices:

1. Wear appropriate laboratory attire, including lab coat, gloves and safety glasses.
2. Do not pipet by mouth, inhale, ingest or allow product to come into contact with open wounds. Wash thoroughly any part of the body which comes into contact with the product.
3. Avoid accidental autoinjection by exercising extreme care when handling in conjunction with any injection device.
4. This product is intended for research use by qualified personnel only. It is not intended for use in humans or as a diagnostic agent. Advanced Targeting Systems is not liable for any damages resulting from the misuse or handling of this product.

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



T47D cells, a human epithelial cell line derived from a mammary gland, were plated at 2500 cells/well/90 ul and incubated overnight. The next day, Saporin (PR-01) and Bombesin-SAP (IT-40) were added in 10 ul volumes at a dosing range of 1 uM-100 fM and 100 nM-10 fM, respectively. The plates incubated for 72 hours at 37°C. To develop, the medium was removed from the plate, and the cells were fixed with 10% TCA for 1 hour at 4°C. The plates were washed 3 times with tap water and allowed to air dry. 50 ul of 0.4% sulforhodamine B/1% acetic acid was added to each well and the plate incubated for 30 minutes at room temperature. The plates were washed 3 times with 1% acetic acid and allowed to air dry. The dye was solubilized with 100 ul of 10 mM unbuffered tris base per well, with 5 minutes of gentle shaking. The plates were read at 564 nm, and data analysis was done with Prism software (GraphPad, San Diego)