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### **Melanopsin-SAP** TARGETED SAP CONJUGATE

*a tool for eliminating cells that express melanopsin in mouse intrinsically photosensitive retinal ganglion cells; targeted via the affinity-purified rabbit polyclonal antibody to mouse melanopsin, eliminated via saporin*

**Catalog Number:** IT-44  
**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.  
**Host:** Rabbit

#### **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.

Melanopsin-SAP specifically eliminates intrinsically photosensitive retinal ganglion cells (ipRGCs) that express melanopsin. The ipRGCs, with their amazing long processes, are involved in the perception of light and dark and are circadian rhythm determinants.

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

This targeted toxin recognizes mouse intrinsically photosensitive retinal ganglion cells (ipRGCs) that express melanopsin. Melanopsin-SAP is a chemical conjugate of an affinity-purified rabbit polyclonal antibody to mouse melanopsin (Cat. #AB-N39) and the ribosome-inactivating protein, saporin (Cat. #PR-01).

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

Melanopsin-SAP specifically eliminates intrinsically photosensitive retinal ganglion cells (ipRGCs) that express melanopsin. Use and effects of Melanopsin-SAP are described in detail in Göz *et al.*<sup>1</sup> **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  $-20^{\circ}\text{C}$  for 1-2 months. For longer term storage store the material at  $-80^{\circ}\text{C}$ . 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):** Melanopsin affinity-purified Rabbit Polyclonal, Rabbit IgG-SAP

**References:**

1. Göz D, Studholme K, Lappi DA, Rollag MD, Provencio I, Morin LP (2008) Targeted destruction of photosensitive retinal ganglion cells with a saporin conjugate alters the effects of light on mouse circadian rhythms. *PLoS ONE* 3(9):e3153.

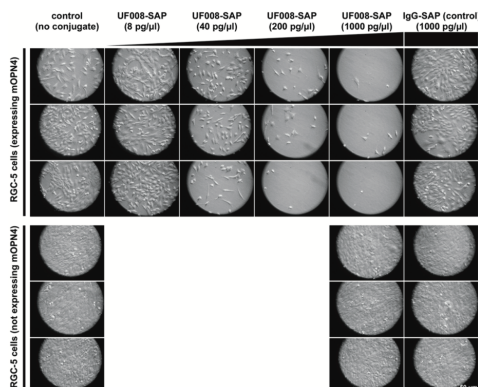
**Safety:**

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.

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A saporin/anti-melanopsin(UF008) conjugate (Cat. #IT-44) destroys cultured RGC-5 cells in a dose-dependent manner. Cultures of RGC-5 cells, either stably expressing or not expressing mouse melanopsin, were exposed to Melanopsin-SAP for 4 days. The concentrations of conjugates are shown above each column and the experiments were done in triplicate. Each panel represents a randomly selected field from a single well.<sup>1</sup>