Advanced Targeting Systems

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FITC-Streptavidin-ZAP

ZAP CONJUGATE

a tool to "piggyback" onto YOUR biotinylated material via Streptavidin

Catalog Number: IT-85
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: There are a growing number of antibody and small molecule therapeutic candidates and this demands a quick and efficient technique to screen for biomarkers that internalize effectively upon binding. FITC-Streptavidin-ZAP (FITC-SA-ZAP) provides for the efficient determination of internalization of cell surface biomarkers upon binding of antibodies or peptides. The construct that makes this method effective was formed by crosslinking a fluorescent reporter, in this case fluorescein (FITC) and Streptavidin (SA) to the ribosome-inactivating protein, saporin (ZAP).
The conjugate used in screening potential targeting agents or cells is a mixture of a biotinylated targeting agent mixed in a 1:1 molar ratio with FITC-labeled Streptavidinylated saporin (FITC-SA-ZAP). The bond between Streptavidin and biotin is rapid and essentially nonreversible, unaffected by most extremes of pH, organic solvents, and denaturing reagents. The method provides a definitive assay readout: fluorescence within 1 hour and cell death in 72 hours. This method is designed for rapid screening, in a quick and reproducible manner, for specificity and internalization in various cell types to explore suitability of targeting agents.

Specificity & Preparation: This conjugate recognizes cells targeted by biotinylated materials. FITC-Streptavidin-ZAP is a chemical conjugate of fluorescein (FITC), Streptavidin (SA) and the ribosome-inactivating protein, saporin (ZAP). FITC-SA-ZAP targets cells that recognize YOUR biotinylated material, eliminates those cells via saporin, and fluoresces the cells via fluorescein isothiocyanate (FITC). This product is routinely tested by flow cytometry and cytotoxicity assay.

Usage: 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. Prepare the solution when ready to use. Mix undiluted FITC-Streptavidin-ZAP with your undiluted biotinylated material at a 1:1 molar ratio and allow to react for 20 min at room temperature.

Storage: Gently spin down material 5-10 seconds in a microfuge before use. The material should be stored at 4°C in undiluted aliquots. Do not use a reducing agent (such as dithiothreitol, beta-mercaptoethanol or ascorbic acid) with this material. It will inactivate the toxin.
If the biotinylated targeting agent is recognized by a human receptor, this material will be toxic to human cells expressing the appropriate receptor. 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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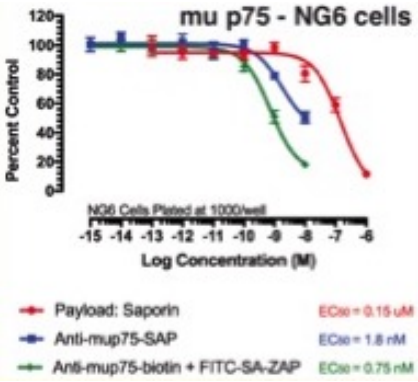
Scan to view all product references.

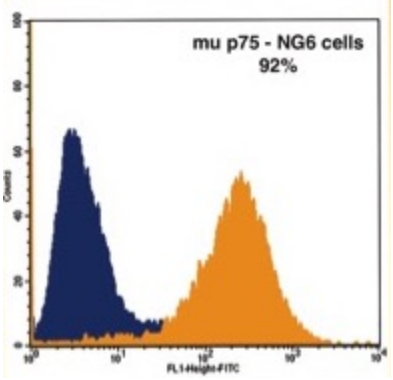
Safety:
Good laboratory technique must be employed for safe handling of this product. This requires observation of the following practices:

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/library/protocols



NG6 cells were plated at 1000 cells/well into 96-well plates and incubated overnight at 37°C to acclimate. Treatment of the cells occurred 24 hours later and unconjugated saporin and directly linked conjugate, anti-mup75-SAP were used as controls. The biotinylated antibody was mixed at a 1:1 molar ratio with FITC-SA-ZAP. Developing reagents XTT/PMS were used to determine cell viability after treatment via absorbance.



NG6 cells were tested to demonstrate the use of FITC-SA-ZAP and biotinylated antibody in a flow cytometry application to detect receptor expression. The Orange peak shows expression of target receptor when stained with 10 ug of biotinylated anti-mu p75 coupled equimolar to FITC-SA-ZAP. A 92% shift is seen versus negative control of cells alone (Blue peak).