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TARGETING
SYSTEMS

6352 CORTE DEL ABETO, STE B
CARLSBAD, CA 92011 USA
01.858.642.1988 • WWW.ATSBIO.COM

Azido-ZAP

TAG-TARGETED TOXIN

terminal azide group-saporin

Catalog Number: BETA-003
Quantity: 25 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 containing a terminal azide group can be combined with an alkyne-containing molecule in a click chemistry reaction. Click chemistry describes a class of chemical reactions that uses bio-orthogonal or biologically unique moities to label and detect a molecule of interest using a two-step procedure. The two-step click reaction involves a copper-catalyzed triazole formation from an azide and an alkyne. The azide and alkyne moieties can be used interchangeably; either one can be used to tag the molecule of interest, while the other is used for subsequent detection. The azides and alkynes are biologically unique, stable, and extremely small.
Click chemistry can be used when methods such as direct labeling or the use of antibodies are not applicable or efficient. The click chemistry label is small enough that tagged molecules (e.g., nucleotides, sugars, and amino acids) are acceptable substrates for the enzymes that assemble these building blocks into biopolymers. The small size of click detection molecules allows them to easily penetrate complex samples, including intact, supercoiled DNA, with only mild permeabilization required.
The characteristics of click reactions include:
• Efficiency—the reaction between the detection moieties is complete in less than 1 hour and does not require extreme temperatures or solvents.
• Stability—the reaction product contains an irreversible, covalent bond.
• Biologically inert—the components of the reaction do not undergo any side reactions.
• Specificity—the reaction between the label and detection tag is selective and specific.
The click chemistry-labeled molecules can be applied to complex biological samples and easily detected with high sensitivity and low background, unlike traditional chemical reactions that use succinimidyl esters or maleimides that target amines and sulfhydryls, which are not unique functional groups.

Specificity and Preparation:
This targeted toxin recognizes molecules containing a free alkyne group. Azido-SAP is a terminal azide group and the ribosome-inactivating protein, saporin. Concentration is 0.9 mg/ml. Average effective molecular weight is 30 kDa.

Usage and Storage:
Azido-ZAP eliminates molecules containing a free alkyne group. All other cells are left untouched. It is not suitable for retrograde transport. **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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References:

1. Salic A, Mitchison TJ. (2008) A chemical method for fast and sensitive detection of DNA synthesis *in vivo*. *Proc Natl Acad Sci U S A* 105(7):2415-2420.
2. Clark PM, Dweck JF, Mason DE, Hart CR, Buck SB, Peters EC, Agnew BJ, Hsieh-Wilson LC. (2008) Direct in-gel fluorescence detection and cellular imaging of O-GlcNAc-modified proteins. *J Am Chem Soc* 130(35):11576-11577.
3. Dieterich DC, Link AJ, Graumann J, Tirrell DA, Schuman EM. (2006) Selective identification of newly synthesized proteins in mammalian cells using bioorthogonal noncanonical amino acid tagging (BONCAT). *Proc Natl Acad Sci U S A* 103(25):9482-9487.

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