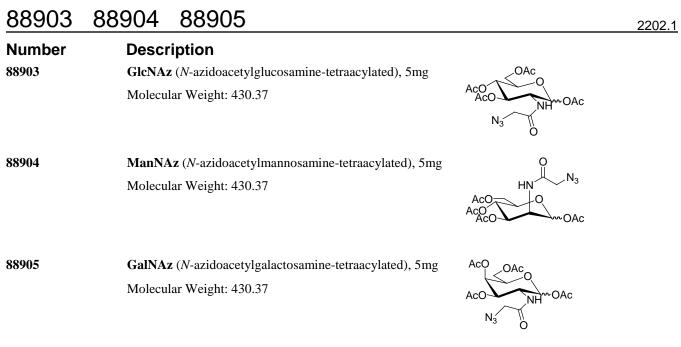
# **INSTRUCTIONS**

# **Azido Sugars**



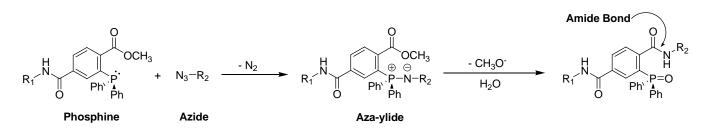


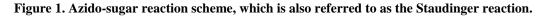
Storage: Upon receipt store at -20°C. Product shipped at ambient temperature.

## Introduction

The Thermo Scientific GlcNAz, ManNAz and GalNAz Reagents are azido sugars that provide a highly specific approach for studying glycoproteins through chemoselective ligation. Reactions using the azido sugars occur between a phosphine and an azide via a Staudinger reaction to produce an aza-ylide intermediate that is trapped to form a stable, covalent amide bond (Figure 1).<sup>1</sup> Because phosphines and azides are absent from biological systems, there is minimal background labeling of macromolecules found in cells or lysates.<sup>2</sup>

There are several classes of glycoproteins grouped by the type of carbohydrate and amino acid linkage site. *N*-linked glycosylation is a modification of asparagine amines, whereas *O*-linked glycosylation occurs through the hydroxyl of serine and threonine residues. The azido sugars are bioorthogonal substitutes for endogenous amino sugars. ManNAz is converted by cells to an azido sialic acid derivative that is used for *N*-linked glycosylation of cell surface proteins. GlcNAz and GalNAz are predominantly used to label the *O*-linked glycosylation (*O*-GlcNAc and *O*-GalNAc).







#### **Important Product Information**

- Azido sugars are stable under typical laboratory lighting conditions; however, avoid exposing reagents to direct sunlight. If possible, avoid reducing agents in reaction buffers, which may interfere with azide stability.
- Reactions with phosphines and azides are more efficient at high concentrations and temperatures (i.e., 23-37°C). Typical reaction times are less than 4 hours; however, incubating for longer can improve reaction efficiency.
- Dissolve azido sugars in a dry water-miscible organic solvent, such as DMSO, before adding to cell culture media. Store DMSO stock solutions at -20°C for up to 6 months.

## Procedure for Labeling Cellular Glycoproteins

- A. Additional Materials Required
- Dry dimethyl sulfoxide (DMSO)
- Mammalian cultured cells

#### **B.** Procedure

- 1. Prepare 10mM of the reagent by dissolving 5mg of the azido-sugar reagent in 1.16mL of DMSO.
- 2. Add the 10mM solution directly to cell culture media for a final concentration of 40µM (1:250 dilution).
- 3. Incubate cells for at least 72 hours in an incubator to incorporate azido sugars into glycoproteins.

#### **Related Thermo Scientific Products**

88901	Biotin-PEG <sub>3</sub> -Phosphine, 10mg
88907	DyLight 488-Phosphine, 1mg
88910	DyLight 550-Phosphine, 1mg
88911	DyLight 650-Phosphine, 1mg
88902	NHS-Azide, 10mg
88900	NHS-Phosphine, 10mg
88906	Sulfo-NHS-Phosphine, 10mg

#### **Cited References**

- 1. Saxon, E. and Bertozzi, C. (2000). Cell surface engineering by a modified Staudinger reaction. Science 287:2007-10.
- 2. Agard, N., et al. (2006). A comparative study of bioorthogonal reactions with azides. ACS Chemical Biology 1(10):644-8.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at <u>www.thermoscientific.com/pierce</u>. For a faxed copy, call 800-874-3723 or contact your local distributor. © 2012 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.