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ProductInformation

N-Azidoacetylgalactosamine, Acetylated
Catalog Number **A7480**
N-Azidoacetylglucosamine, Acetylated
Catalog Number **A7355**
N-Azidoacetylmannosamine, Acetylated
Catalog Number **A7605**

Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Sigma-Aldrich's set of azido sugars consists of three peracetylated azide monosaccharides:

N-Azidoacetylgalactosamine, Acetylated (Ac_4GalNAz)
N-Azidoacetylglucosamine, Acetylated (Ac_4GlcNAz)
N-Azidoacetylmannosamine, Acetylated (Ac_4ManNAz)

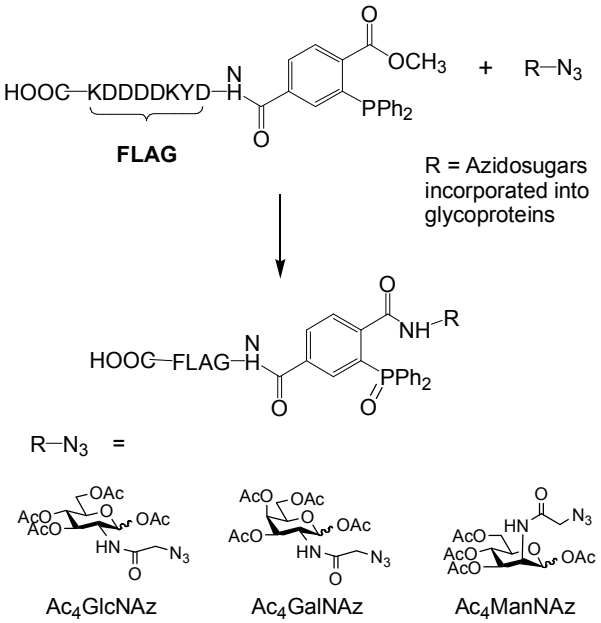
They are designed for use with the GlycoProfile™ FLAG® Phosphine Conjugate, Catalog Number GPHOS1.

Mammalian cells incorporate these azide sugars into both cell surface and internal glycans using existing biosynthetic pathways. For example, Ac_4ManNAz, a precursor to sialic acid, is incorporated into the sialic acid biosynthesis pathway.[1-3]

The acetyl groups increase cell permeability and allow the unnatural sugars to easily pass through the cell membrane. Carboxyesterases remove the acetyl groups once the monosaccharide is in the cell. After incorporation, the azide group, which is orthogonal to the glycan linkage, is then available for reaction with the phosphine group of the FLAG phosphine conjugate. The azide-phosphine linkage is preferential, with no comparable functional groups inherent in cells or tissues that would compete with the reactive groups. The reaction occurs at neutral pH, 7.0–7.4, aqueous conditions and is amenable to labeling live cells.[1,4]

The azide-phosphine reaction, which produces an aza-ylide intermediate, is termed the Staudinger reaction. This reaction has been modified such that the aza-ylide intermediate rearranges in aqueous media to produce an amide linkage and phosphine oxide. The modified reaction is designated as the Staudinger Ligation. See Figure 1.[1]

Figure 1.
Representation of the Staudinger Ligation



This conjugation chemistry has many applications in the field of biochemistry including monitoring carbohydrate specific responses, delivery of therapeutic agents, cell line response, and tissue engineering. The use of the FLAG phosphine conjugate allows use of different detection techniques, including, but not limited to, chemiluminescence. In addition, the FLAG peptide sequence allows for purification of the labeled glycans by immunoprecipitation (IP).

Reagents and equipment required but not provided

- Ethanol, 200 proof (absolute), for molecular biology, Catalog Number E7023
- Glycoprofile™ FLAG® Phosphine Conjugate, Catalog Number GPHOS1
- Phosphate buffered saline (PBS), Catalog Number P5368
- Six well plate
- Centrifuge and centrifuge tubes

Products required for specific procedures are listed with catalog numbers in the described methods.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

1. 300 μM Azido Sugar Solution – Dissolve 1 mg of the azido sugar with 7.75 ml of ethanol. For smaller volumes, weigh out the appropriate amount and adjust the volume of ethanol proportionally. Tightly cap the vial to limit evaporation. Ac_4ManNAz is water-soluble and may be dissolved directly in the cell culture medium at a concentration of 50 μM. **Note**: These solutions should be used within **24 hours** of reconstitution.

2. 250 μM FLAG Phosphine Solution – The contents of the entire vial (1 mg) may be reconstituted with 2.96 ml of phosphate buffered saline (PBS). For smaller volumes, weigh out the appropriate amount of material and adjust the volume of PBS proportionally.
   Note: Prepare this solution immediately prior to harvesting the cells for incubation. This solution must be used within **4 hours** of reconstitution.

Storage/Stability

The azido sugars ship at room temperature and storage at 2–8 °C is recommended.

Procedures

These procedures are intended as guidelines and may not be appropriate for all situations. These procedures have been tested with several cell lines, CHO, HeLa, and Jurkat, and may be appropriate for other mammalian cell lines.[5,6]

Incubation of Cells

1. Add 500 μL of the 300 μM Azido Sugar Solution to each well of a six well plate.
2. Allow the ethanol to evaporate to dryness in a biological hood before the addition of cells.
3. Seed each well of the plate with 3 ml of medium containing 2 x 10^5 cells/ml, for a total of 6 x 10^5 cells. The final azido sugar concentration will be 50 μM after the addition of cells. Place the plate in a CO_2 incubator at 37 °C for 48–72 hours.
4. After incubation, transfer the contents of each well to a separate 15 ml centrifuge tube. Collect the cells after centrifugation at 100 × *g* for 5 minutes.
5. After decanting the medium, wash the cells twice with PBS with a centrifugation after each wash.
6. Incubate the cells overnight at 2–8 °C in the 250 μM FLAG Phosphine Solution. (200 μL/1.5 × 10^6 cells).
   Notes:
   - For labeling cytosolic proteins it is recommended that the cells be lysed prior to the addition of the 250 μM FLAG Phosphine Solution.
   - For some applications the labeling step can be accelerated by incubating the cells at 37 °C for 2 hours.

Lysis of Cells with RIPA Buffer

<u>Reagents recommended, but not provided</u>

- Protease Inhibitor Cocktail, for use with mammalian cell and tissue extracts, Catalog Number P8340
- RIPA Buffer, Catalog Number R0278

7. Pellet the cells with centrifugation at 200 × *g* for 5 minutes and aspirate the FLAG Phosphine Solution.
8. Wash the cells twice with PBS, with a centrifugation after each wash. Decant the PBS and resuspend the cells in RIPA Buffer, containing Protease Inhibitor Cocktail, for use with mammalian cell and tissue extracts
9. Vortex the cells, place on ice for 5 minutes, and vortex again.
10. Centrifuge the cell suspension at 9000 × *g* for 10 minutes to pellet the cell debris.
11. Transfer the supernatant to a clean tube.

Western Blot Immunostaining [6-8]

Note: This procedure uses chemiluminescent detection with Chemiluminescent Peroxidase Substrate-3. Dilutions of the primary antibodies may require optimization when using other substrates or conditions.

Reagents recommended, but not provided

- Chemiluminescent Peroxidase Substrate-3 (CPS3), Catalog Numbers CPS350, CPS3100, or CPS3500
- Kodak® BioMax™ light film, Catalog No. Z373494
- Monoclonal ANTI-FLAG® M2-Peroxidase (HRP), Catalog Number A8592
- Ponceau S solution, Catalog Number P7170
- Tris Buffered Saline, pH 8.0, with 3% nonfat Milk, Catalog Number T8793
- Tris Buffered Saline, with TWEEN® 20, pH 8.0, (TBST), Catalog Number T9039.

1. Use a standard SDS-PAGE procedure to separate the components of each sample lysate, including the glycoproteins labeled with the FLAG peptide sequence.
2. Transfer the proteins from the gel to a nitrocellulose membrane, or Immobilon-P™ or other polyvinylidene difluoride (PVDF) membrane. The PVDF membrane may provide greater downstream sensitivity. The proteins may be visualized using Ponceau S solution.
3. Block the blot with at least 0.5 ml/cm^2 of Tris Buffered Saline, pH 8.0, with 3% nonfat milk for 1 hour at room temperature, employing gentle agitation.
4. Remove the blocking agent and add 25 ml of Monoclonal ANTI-FLAG M2-Peroxidase (HRP), diluted 1:1000 in TBS with 3% nonfat milk. Incubate for 1 hour at room temperature with gentle agitation.
5. Decant the ANTI-FLAG M2 antibody solution. Wash the membrane three times for 5 minutes each with at least 0.5 ml/cm^2 of Tris Buffered Saline, with TWEEN® 20, pH 8.0 (TBST). Use gentle agitation during the washes.
6. Develop the blot using Chemiluminescent Peroxidase Substrate-3 (CPS3) or an equivalent reagent for 5 minutes. Do not agitate the blot during this step. Drain briefly and wrap in plastic film.
7. Expose BioMax light film to the blot for a duration of several seconds to 10 minutes. A quick exposure of 10–30 seconds is recommended to determine the optimal exposure time needed. If the signal is too intense even at the short exposure times, allow the signal to decay over a 1–8 hour period and then re-expose the film.

Immunoprecipitation (IP)

Reagents recommended, but not provided

- Monoclonal ANTI-FLAG BioM2, Catalog Number F9291
- Streptavidin Agarose, Catalog Number S1638.

It has been demonstrated that immunoprecipitation of glycoproteins labeled with the FLAG peptide sequence using Monoclonal ANTI-FLAG M2 antibody is feasible. To allow an optimal interaction it is recommended that a soluble ANTI-FLAG M2 antibody be used to capture the labeled glycoprotein(s). The glycoprotein mixture may require denaturing for a proper presentation of the FLAG epitope resulting in a more efficient immuno-precipitation. In one example Monoclonal ANTI-FLAG BioM2 was used to bind to the glycoconjugate followed by precipitation with Streptavidin Agarose.

Immunocytochemistry

Reagents recommended, but not provided

- Monoclonal ANTI-FLAG M2-FITC Conjugate, Catalog Number F4049

It has been demonstrated that cell surface glyco-proteins labeled with the FLAG peptide sequence can be stained using Monoclonal ANTI-FLAG M2-FITC. Optimal results were obtained for adherent cell lines grown and labeled on glass cover slips plated in a six well plate format. They were labeled for 2 hours at 37 °C with the FLAG Phosphine conjugate. The cell surface glyco-proteins can subsequently be stained with Monoclonal ANTI-FLAG M2-FITC.

References

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