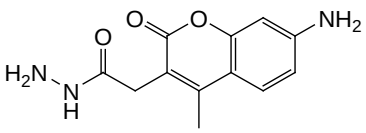
INSTRUCTIONS

AMCA-Hydrazide

33015

0088.2

Number	Description
33015	**AMCA-Hydrazide,** 5mg Molecular Weight: 247 Excitation: 345-350nm Emission: 440-460nm (blue)



Storage: Upon receipt store at 4°C protected from moisture. Reagent shipped at ambient temperature.

Introduction

The Thermo Scientific AMCA-Hydrazide is a carbonyl-reactive fluorophore for labeling antibodies, proteins and other molecules. AMCA exhibits a large Stokes shift, which decreases potential interference from Rayleigh and Raman scatter and other fluorescing substances, allowing easy discrimination of the label after excitation. The brilliant blue fluorescence is easily visualized, does not readily photobleach, and is suitable for double labeling with red or green fluorophores such as fluorescein and lissamine.

Hydrazide groups form hydrazone bonds upon reaction with carbonyls (aldehydes and ketones), which occur at the reducing end of polysaccharides or may be created by oxidation of carbohydrate groups in glycoproteins and other molecules. Sialic acid is a common sugar component of protein polysaccharides, and it is easily oxidized with 1mM sodium *meta*-periodate ($NaIO_4$). Other sugar groups can be oxidized effectively with 5-10mM sodium *meta*-periodate. For glycoproteins, sugar oxidation enables labeling directed away from polypeptide domains that are important for protein function. For example, most polyclonal antibodies are glycosylated in regions other than antigen-binding sites, enabling them to be labeled with hydrazide reagents without adversely affecting their function in immunoassays.

AMCA-Hydrazide also can be reacted with carboxyl groups using the carbodiimide EDC (Product No. 22980). EDC activates carboxyl groups to bind to the $–NH_2$ group of the labeling reagent, forming an amide linkage. Using EDC may result in some polymerization if the target molecule has both carboxyls and primary amines on its surface. Decreasing the amount of EDC and/or increasing the amount of labeling reagent used in the reaction can minimize polymerization. For a complete EDC protocol, consult instructions for EDC or for the related biotin-hydrazide reagents (e.g., Product No. 21360).

Important Product Information

- The glycoprotein or other material to be labeled with AMCA-Hydrazide must have reactive aldehyde or ketone groups (carbonyls). These groups may exist at the reducing end of polysaccharides. To create additional carbonyls, oxidize sugar groups using either a specific oxidase, such as galactose oxidase, or 1-10mM sodium *meta*-periodate ($NaIO_4$; Product No. 20504). Oxidation with periodate is most efficient in acidic conditions (e.g., 0.1M sodium acetate, pH 5.5), although neutral buffers such as phosphate-buffered saline can be used. Avoid buffers that contain reducing agents such as 2-mercaptoethanol or dithiothreitol, which will interfere with oxidation. Additionally, avoid materials that can be oxidized, such as glycerol, which will complete with the reaction.
- Hydrazides react with carbonyls most efficiently in amine-free, near-neutral conditions (pH 6.5-7.5). If oxidation is performed in acidic conditions, buffer exchange by dialysis or gel filtration (see Related Thermo Scientific Products) into neutral buffer may be necessary to obtain efficient hydrazide conjugation. Avoid Tris or other primary amine-containing buffers during glycoprotein oxidation and the hydrazide reaction because they can react with the aldehyde groups, competing with the intended reaction to the hydrazide.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce

Example Procedure for Glycoprotein (e.g., Antibody) Labeling

Note: For best results, optimize the molar ratio of reagent and glycoprotein by empirical testing.

A. Materials Required

- AMCA-Hydrazide Solution: Prepare a 2mM solution by dissolving 0.5mg AMCA-Hydrazide in 1mL dimethyl formamide (DMF, Product No. 20673) in a foil-wrapped or amber vial. Prepare a volume sufficient to achieve the desired final concentration in step B.4. Excess (unused) dissolved reagent may be stable, but this has not been verified. Protect the solid reagent and all solutions made from the fluorophore from light to avoid photodecomposition.
- Oxidation Buffer: 0.1M sodium acetate buffer, pH 5.5
- Sodium *meta*-periodate (Product No. 20504) solution: 20mM sodium *meta*-periodate in Oxidation Buffer. Prepare solution immediately before use in a foiled-wrapped or amber vial (i.e., protected from light).
- Coupling Buffer: 0.1M sodium phosphate, 0.15M NaCl, pH 7.2 (Phosphate-buffered saline, PBS, Product No. 28372) or other neutral or slightly alkaline, non-amine buffer
- Glycoprotein Solution: 2mg/mL of glycoprotein in Oxidation Buffer
- Dialysis Cassette (e.g., Thermo Scientific Slide-A-Lyzer Dialysis Cassette Kit, 10K MWCO, 0.5-3mL, Product No. 66382) or Desalting Column (e.g., Thermo Scientific Zeba Spin Desalting Columns, 5 × 5mL columns for desalting 0.5-2mL samples, Product No. 89891)

B. Procedure

1. Add 1mL of cold sodium *meta*-periodate solution to 1mL of cold glycoprotein solution; mix well and then protect reaction vessel from light and incubate mixture for 30 minutes on ice or at 4°C.

   Note: To oxidize only sialic acid groups, add 50µL of sodium *meta*-periodate instead of 1mL (results in 1mM periodate final concentration rather than 10mM).

2. Remove excess periodate and exchange the sample buffer by dialysis against coupling buffer or by gel filtration through a desalting column that has been equilibrated with coupling buffer.
3. Add 50-100µL prepared AMCA-Hydrazide Solution per milliliter oxidized glycoprotein solution; mix for 2 hours at room temperature protected from light.

   Note: Optimal AMCA-Hydrazide concentration and reaction conditions depend on target protein and downstream application and must be determined empirically.

4. Separate the AMCA-labeled protein from excess non-reacted AMCA reagent by dialysis or gel filtration (desalting column). Minimize exposure to light; store conjugate in a manner appropriate for the protein and fluorophore stability.

   Note: To assess the level of fluorophore modification (F/P ratio), consult Tech Tip #31: Calculate dye:protein (F/P) ratios, which is available from the Technical Resources section of the website.

Related Thermo Scientific Products

20036	**Bioconjugate Techniques,** by Greg T. Hermanson, 1996, Academic Press, softcover, 785 pages
21360	**Biotin-PEO$_4$-Hydrazide,** 100mg
20673	**Dimethylformamide (DMF), Sequencing grade,** 50mL
66382	**Slide-A-Lyzer® Dialysis Cassette Kit, 10K MWCO, 3mL**
89891	**Zeba™ Spin Desalting Columns, 7K MWCO, 5mL,** 5/pkg

General References

Khalfan, H., *et al*. (1986). Aminomethyl coumarin acetic acid: a new fluorescent labeling reagent for proteins. *Histochem J* **18:**497-9.

O'Shannessy, D. J., *et al*. (1984). A novel procedure for labeling immunoglobulins by conjugation to oligosaccharide moieties. *Immuno Lett* **8:**273-7.

Wade, D. P., *et al*. (1985) Detection of the low density-lipoprotein receptor with biotin-low density lipoprotein. *Biochem J* **229:**785-90.

O'Shannessy, D. J., *et al*. (1987). Quantitation of glycoproteins on electroblots using the biotin-streptavidin complex. *Anal Biochem* **163:**204-9.

Roffman, E. (1986). Selective labeling of functional groups on membrane proteins or glycoproteins using reactive biotin derivatives of 125I-streptavidin. *Biochem Biophys Res Comm* **136:**80-5.

Rosenberg, M. B., *et al*. (1986). Receptor binding activities of biotinylated derivatives of B-nerve growth factor. *J Neurochem* **46:**641-8.

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 *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.