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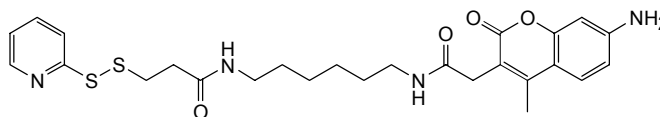
**Number**

33020

**Description**

AMCA-HPDP, 5 mg

Molecular Weight: 528.69



Excitation/Emission Wavelengths: 345-350/440-460 nm (blue)

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

**Introduction**

AMCA-HPDP (*N*-[6-7-amino-4-methylcoumarin-3-acetamido] hexyl]-3'-[2'-pyridylthio] propionamide) is a sulfhydryl-reactive fluorophore for labeling antibodies, proteins and other molecules. The 2-pyridylthio group of AMCA-HPDP reacts with sulfhydryls optimally at pH 7-8. The reaction results in displacement of a pyridine-2-thione group, the concentration of which can be determined by measuring the absorbance at 343 nm. The resulting disulfide bond can be reduced to regenerate the protein in its original, unmodified form. Additionally, AMCA exhibits a large Stokes shift, which decreases potential interference from Rayleigh and Raman scatter along with other fluorescing substances allowing easy discrimination of the label after excitation. The brilliant blue fluorescence is easily visualized, does not readily photobleach, and is well suited for double and triple labeling with fluorochromes such as fluorescein, lissamine and others that are red or green.

**Important Product Information**

- Molecules for labeling with AMCA-HPDP must have free -SH group(s) available. Some sulfhydryl-containing peptides and proteins may oxidize in solution and form disulfide bonds, which cannot react with maleimides. Disulfide bonds can be reduced to produce free sulfhydryls. After reduction, most reducing reagents must be removed before conjugation. The Reduce-Imm™ Reducing Kit (Product No. 77700) and Immobilized TCEP Disulfide Reducing Gel (Product No. 77712) enables peptide or protein reduction while recovering the sample in the absence of reducing agents.
- As an alternative to disulfide reduction, sulfhydryls can be introduced via amine modification using *N*-succinimidyl *S*-acetylthioacetate (SATA, Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101).
- Avoid sulfhydryl-containing components during conjugation, as these will react with the pyridyl-disulfide group of the reagent thereby inhibiting and reducing conjugation efficiency of the intended molecule.

**Example Procedure for IgG Reduction and Labeling**

The following protocol is an example application for this product. Specific applications require optimization. In this method, whole IgG is reduced with 2-MEA, which cleaves disulfide bonds between the antibody heavy chains while preserving disulfide bonds between the heavy and light chains. During reduction, the absolute concentration of 2-MEA is more critical than antibody concentration as 1-10 mg IgG can be effectively reduced with 50 mM 2-MEA. To prevent metal-catalyzed oxidation of sulfhydryls, EDTA is included in buffers. The protocol can be modified for other molecules.

**A. Additional Materials Required**

- 2-Mercaptoethylamine•HCl (2-MEA, Product No. 20408)
- 0.5 M EDTA

**Warranty:** Pierce Biotechnology products are warranted to meet stated product specifications and to conform to label descriptions when stored and used properly. Unless otherwise stated, this warranty is limited to one year from date of sale when used according to product instructions. Pierce Biotechnology's sole liability for the product is limited to replacement of the product or refund of the purchase price. Unless otherwise expressly authorized in writing by Pierce Biotechnology, Pierce products are supplied for Research Use Only and are intended to be used by a technically qualified individual. Pierce Biotechnology's quality system is certified to ISO 9001. Pierce Biotechnology products are not produced in accordance with FDA's current Good Manufacturing Practices. Pierce Biotechnology strives for 100% customer satisfaction. If you are not satisfied with the performance of a Pierce Biotechnology product, please contact Pierce Biotechnology or your local distributor.

- 1 M Sodium phosphate, pH 6.0
- Phosphate buffered saline (PBS): 0.1 M phosphate, 0.15 M NaCl, pH 7.2, such as BupH™ Phosphate Buffered Saline Packs (Product No. 28372) or other buffer at pH 6.5-7.5
- 37°C incubator or water bath
- Desalting column, such as D-Salt™ Dextran Desalting Columns (Product No. 43230) or Zeba™ Desalt Spin Columns, 5 ml (Product No. 89892) for removing 2-MEA. For removal of excess AMCA, either a desalting column or a Slide-A-Lyzer® Dialysis Cassette may be used.

## B. Material Preparation

- Reducing Buffer: Prepare 1 ml of buffer by combining 100 µl of 1 M sodium phosphate pH 6.0, 5 µl of 0.5 M EDTA and 900 µl ultrapure water.
- Conjugation Buffer: Add 20 µl of 0.5 M EDTA to 10 ml of PBS for each 10 ml of Conjugation Buffer required.
- IgG Solution: Dissolve 2.5 mg IgG in 1 ml of Reducing Buffer.

## C. Reduction of IgG Disulfide Bonds

1. Add the 1 ml IgG Solution to a 6 mg vial of 2-MEA and gently shake vial to dissolve.
2. Incubate reaction for 90 minutes at 37°C.
3. Cool the solution to room temperature.
4. Remove 2-MEA from the reduced antibody using a desalting column equilibrated with Conjugation Buffer.
5. Proceed immediately to Section D to minimize sulfhydryl oxidation.

## D. Labeling of Reduced IgG

**Note:** Upon reduction or modification of the protein it is essential to remove the excess reducing or modification reagent by desalting before reaction with AMCA-HPDP.

1. Dissolve 1 mg AMCA-HPDP in 1 ml of DMF.
2. Add 20 µl of the dye to the tube containing the reduced IgG solution and mix well.
3. Allow the reaction to proceed for 1 hour at room temperature.
4. Remove non-reacted dye from the antibody by desalting or dialysis.
5. Store labeled antibody protected from light at 4°C for up to one month. Alternatively, store labeled antibody in single-use aliquots at -20°C.

**Note:** The extinction coefficient of pyridine-2-thione at 343 nm is  $8.08 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ , such that its concentration is described by the following equation:

$$\text{Molar concentration} = \frac{\text{Absorbance at 343 nm}}{\text{Molar extinction coefficient} \times \text{Path length}} = \frac{A_{343}}{8080}$$

## Troubleshooting

Problem	Cause	Solution
The protein was not labeled	Substance interfered with the reaction or incorrect reaction conditions	Ensure that the Conjugation Buffer is at pH 6-7 and does not contain free thiols, such as reducing agents
	There are no free sulfhydryls available on the protein	Reduce existing disulfide bonds to generate free sulfhydryls, or introduce sulfhydryls with Traut's Reagent or SATA

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## Additional Information

Please visit the Pierce web site for additional information relating to this product including the following items:

- Tech Tip: Protein stability and storage
- Tech Tip: Calculate Dye/Protein (F/P) Molar Ratios
- Tech Tip: An overview of dialysis
- Tech Tip: Extinction coefficients guide
- Tech Tip Protocol: Modify and label oligonucleotide 5' phosphate groups

## Related Pierce Products

26101	Traut's Reagent, 500 mg
26102	SATA ( <i>N</i> -succinimidyl <i>S</i> -acetylthioacetate), 50 mg
26103	Hydroxylamine Hydrochloride, 25 g
77712	Immobilized TCEP Disulfide Reducing Gel, 5 ml
20291	No-Weigh™ Dithiothreitol (DTT), 48 × 7.7 mg microtubes
20408	2-Mercaptoethylamine•HCl, 6 × 6 mg
20490	TCEP•HCl, 1 g
77700	Reduce-Imm™ Reducing Kit, for simultaneous reduction and recovery of peptides
46200	DyLight™ 547 NHS Ester, 1 mg, for labeling at primary amino groups
46205	DyLight™ 647 NHS Ester, 1 mg, for labeling at primary amino groups

Current versions of product instructions are available at [www.piercenet.com](http://www.piercenet.com). For a faxed copy, call 800-874-3723 or contact your local distributor.

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