Revised: May 2, 2022

Product Information

Aminooxy Conjugates

Unit Size: 5 mg

Product List

Cat no.	Product	Ex/Em (nm)	MW (free acid form)
90103	Aminooxy-5(6)-FAM	494/521 (pH 9)	563
90104	Aminooxy-5(6)-ROX	568/595 (MeOH)	936
90105	Aminooxy-5(6)-TAMRA	540/565 (MeOH)	816
96009	Cyanine 555 Aminooxy	555/565 (Water)	931
96008	Cyanine 647 Aminooxy	650/665 (Water)	957
90113	Aminooxy-biotin	---	516

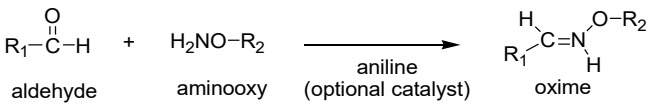
Storage and Handling

Aminooxy-biotin (90113) may be stored at 4°C with desiccant, protected from light. All other aminooxy conjugates may be stored at -20°C with desiccant, protected from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

Stock solutions may be prepared in DMF or DMSO. Solutions can be aliquoted and stored with desiccant and protected from light at 4°C to -20°C, for up to 12 months.

Product Description

Aminooxy (aka hydroxylamine or aminooxyacetamide) reagents can be used to label polysaccharides, glycoproteins, and abasic sites in damaged DNA. Aminooxy reagents react with aldehydes or ketones to form a stable oxime linkage under mild conditions (see reaction scheme below). The reaction is fast and can be further accelerated by adding aniline as a catalyst (1-3). Thus, aminooxy reagents are superior to hydrazide reagents, which also react with aldehydes or ketones but form unstable hydrazone linkages.



The unique reactivity of aminooxy groups provide a convenient and rapid way to label glycoproteins with a detectable tag, such as biotin or a fluorescent dye, under mild conditions. The labeling process involves two steps, first introducing aldehyde groups into the glycoproteins by mild periodate oxidation and then treating the functionalized proteins with an aminooxy reagent. The use of aminooxy labeling has been described in numerous publications. As an example, aminooxy-biotin has been used to efficiently label cell-surface sialic acid-containing glycans on living animal cells. The method has been demonstrated to be superior to "Click" chemistry or the Staudinger chemistry-based bioorthogonal ligation method (4).

Biotium offers a series of aminooxy reagents labeled with biotin, commonly used fluorescent dyes, and our exceptionally bright and photostable CF® Dyes (see Related Products). Biotium also offers CF®DI dye aminooxy conjugates which are designed to match for both charge and molecular weight of labeled proteins (see Related Products).

For more information on these products, particularly on our outstanding CF® Dyes, please visit www.biotium.com

Molecular Information

Product	Chemical Formula	Color and Form
Aminooxy-5(6)-FAM	$C_{26}H_{21}F_3N_2O_9$	Yellow solid
Aminooxy-5(6)-ROX	$C_{45}H_{51}F_6N_5O_{10}$	Dark purple solid
Aminooxy-5(6)-TAMRA	$C_{36}H_{39}F_6N_5O_{10}$	Dark red solid
Cyanine 555 Aminooxy	$C_{38}H_{48}F_6N_4O_{12}S_2$	Dark red solid
Cyanine 647 Aminooxy	$C_{40}H_{50}F_6N_4O_{12}S_2$	Dark blue solid
Aminooxy-biotin	$C_{19}H_{32}F_3N_5O_6S$	Off-white solid

For chemical structures see Figures 1-6.

General protocol for labeling protein aldehyde or protein ketone with aminooxy conjugate

Materials required but not provided

- DMSO or DMF (DMSO Cat no. 90082)
- Aniline 10X, Acetate Buffer (Cat no. 91057)
- 1X Phosphate Buffered Saline (PBS)
- Ultrafiltration Vials (see Related Products)

Procedure

1. Prepare a 5 mM stock solution of the aminooxy conjugate in DMSO or DMF.
2. Prepare a stock solution of protein aldehyde or protein ketone in 1X PBS buffer, preferably reaching a concentration between 20 uM-100 uM. Lower concentrations may result in poor protein recovery yield or inefficient labeling.

   Note: For labeling glycoproteins, perform the steps in the Additional Protocol for Glycoprotein Oxidation below.
3. Add 50 molar equivalents of aminoooxy reagent to the solution prepared in step 2. For example, if you have 100 uL of protein aldehyde or protein ketone at 50 uM (amount of protein aldehyde or protein ketone is 5 nmol) add 250 nmol of aminooxy conjugate (which means 50 uL of 5 mM stock solution).
4. Initiate the ligation by adding 1/10 volume of aniline acetate catalyst (Cat no. 91057). For example, if the mixture from step 3 is 150 uL in total, add 15 uL of catalyst.
5. Vortex the solution and allow the reaction to proceed at room temperature with agitation for 2 hours for protein aldehyde or 5 to 10 hours for protein ketone in the dark.
6. Purify the aminooxy-labeled protein conjugate by Sephadex® G25 column or centrifugal protein concentrator. Biotium offers ultrafiltration vials with molecular weight cut-off of 10 kDa or 3 kDa (see Related Products). To remove free dye by ultrafiltration, choose a molecular weight cut-off that is at least three times larger than your labeled protein, and follow the instructions provided with the ultrafiltration vial.
7. Confirm the formation of product by SDS-PAGE analysis, MALDI-MS analysis, or LC-MS analysis.

Additional Protocol for Glycoprotein Oxidation

For labeling glycoproteins with aminooxy conjugate, oxidation must be performed to convert glycoproteins to protein aldehydes before labeling.

1. Prepare 10X reaction buffer: 1 M sodium acetate; 1.5 M NaCl in DI water, pH 5.5.

2. Prepare 100 mM sodium periodate ($NaIO_4$) stock solution in DI water.
3. Prepare protein solution in 1X PBS buffer, preferably reaching a concentration between 20 uM -100 uM.
4. Add 1/10 volume of 10X reaction buffer prepared in step 1 and 1/10 volume of $NaIO_4$ stock solution prepared in step 2 to the protein solution prepared in step 3. For example, if you have 100 uL of protein solution, add 10 uL of 10X reaction buffer and 10 uL of $NaIO_4$ stock solution.
5. Incubate for 10 min at room temperature or 30 min on ice.
6. Add ethylene glycol to a final concentration of 100 mM to quench the periodate. Incubate 10 min, RT.

   Note: The molarity of pure ethylene glycol is 14.5 M. Add 0.69 uL of ethylene glycol to each 100 uL of reaction mixture. Alternatively, make 1M ethylene glycol stock solution in DI water and add 1/10 volume.
7. Proceed to the aminooxy labeling protocol step 3.

References

1) Bioconjug. Chem. 19(12), 2543(2008); 2) Am. Chem. Soc. 128(49), 15602(2006); 3) Chem. Int. Ed. Engl. 45(45), 7581(2006); 4) Nature Methods. 6(3), 207(2009).

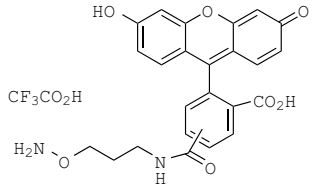


Figure 1. Aminooxy-5(6)-FAM

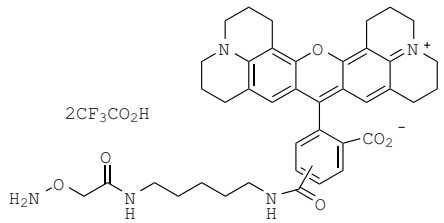


Figure 2. Aminooxy-5(6)-ROX

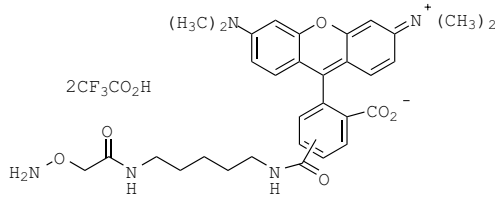


Figure 3. Aminooxy-5(6)-TAMRA

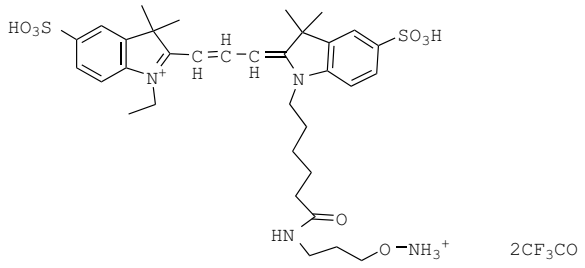


Figure 4. Cyanine 555 Aminooxy

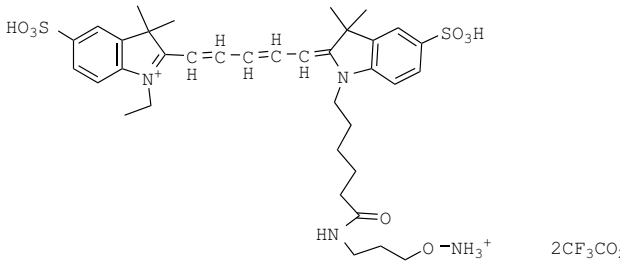


Figure 5. Cyanine 647 Aminooxy

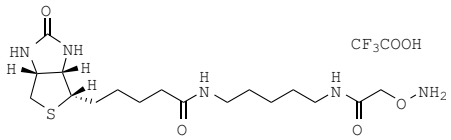


Figure 6. Aminooxy-biotin

Related Products

Catalog number	Product
90082	DMSO, Anhydrous
91057	Aniline, 10X in Acetate Buffer
22014	Ultrafiltration Vial, 10K MWCO (5 per pack)
22018	Ultrafiltration Vial, 3K MWCO (5 per pack)
92050	CF®350 Aminooxy
92055	CF®405S Aminooxy
92056	CF®405M Aminooxy
92051	CF®488A Aminooxy
92057	CF®568 Aminooxy
92052	CF®594 Aminooxy
92053	CF®633 Aminooxy
92058	CF®640R Aminooxy
92059	CF®660R Aminooxy
92054	CF®680R Aminooxy
92177	CF®488DI Aminooxy
92178	CF®555DI Aminooxy
92179	CF®647DI Aminooxy
29030... 29086	CF® Dye Streptavidin Conjugates
20203... 20501	Biotin Monoclonal Mouse Antibody (3D6.6)
BNUB0400	Biotin Monoclonal Mouse Antibody (Hyb8)
BNUB2032	Biotin Recombinant Monoclonal Rabbit Antibody (BTN/2032R)

Please visit our website at www.biotium.com to view our full selection reactive dyes, labeled antibodies, labeling kits, and other conjugates featuring our bright and photostable CF® Dyes and other widely used fluorescent dyes.

CF dye technology is covered by pending US and international patents. Sephadex is a registered trademarks of GE Healthcare.