

Product Information

Aminoxy Conjugates

Unit Size: 5 mg

Product List

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

Note: Cyanine 555 and Cyanine 647 are structurally identical to Cy@3 and Cy@5 dyes from GE Healthcare.

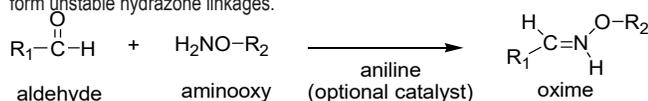
Storage and Handling

Aminoxy-biotin (90113) may be stored at 4°C with desiccant, protected from light. All other aminoxy 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

Aminoxy (aka hydroxylamine or aminoxyacetamide) reagents can be used to label polysaccharides, glycoproteins, and abasic sites in damaged DNA. Aminoxy 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, aminoxy reagents are superior to hydrazide reagents, which also react with aldehydes or ketones but form unstable hydrazone linkages.



The unique reactivity of aminoxy 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 aminoxy reagent. The use of aminoxy labeling has been described in numerous publications. As an example, aminoxy-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 aminoxy 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 aminoxy 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
Aminoxy-5(6)-FAM	C ₂₆ H ₁₂₁ F ₃ N ₂ O ₉	Yellow solid
Aminoxy-5(6)-ROX	C ₄₅ H ₅₁ F ₆ N ₅ O ₁₀	Dark purple solid
Aminoxy-5(6)-TAMRA	C ₃₆ H ₃₉ F ₆ N ₅ O ₁₀	Dark red solid
Aminoxy-biotin	C ₁₉ H ₃₂ F ₃ N ₅ O ₆ S	Off-white solid
Cyanine 555 Aminoxy	C ₃₈ H ₄₈ F ₆ N ₄ O ₁₂ S ₂	Dark red solid
Cyanine 647 Aminoxy	C ₄₀ H ₅₀ F ₆ N ₄ O ₁₂ S ₂	Dark blue solid

General protocol for labeling protein aldehyde or protein ketone with aminoxy 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 aminoxy 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 aminoxy 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 aminoxy 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 aminoxy-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 aminoxy 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 (NaIO₄) stock solution in DI water.

- Prepare protein solution in 1X PBS buffer, preferably reaching a concentration between 20 μM -100 μM .
- 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 μL of protein solution, add 10 μL of 10X reaction buffer and 10 μL of NaIO_4 stock solution.
- Incubate for 10 min at room temperature or 30 min on ice.
- 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 μL of ethylene glycol to each 100 μL of reaction mixture. Alternatively, make 1M ethylene glycol stock solution in DI water and add 1/10 volume.

- Proceed to the aminoxy labeling protocol step 3.

References

- 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).

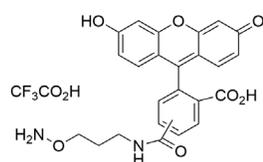


Figure 1. Aminoxy-5(6)-FAM

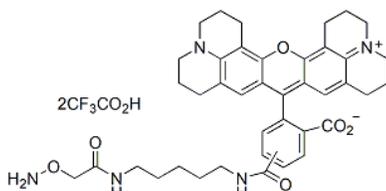


Figure 2. Aminoxy-5(6)-ROX

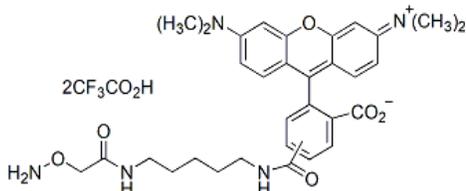


Figure 3. Aminoxy-5(6)-TAMRA

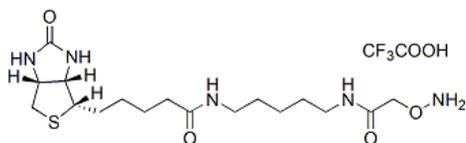


Figure 4. Aminoxy-biotin

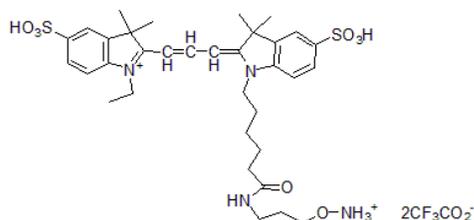


Figure 5. Cyanine 555 Aminoxy

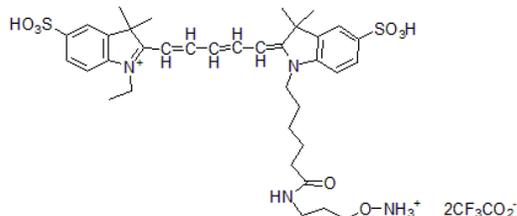


Figure 6. Cyanine 647 Aminoxy

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 Aminoxy
92055	CF@405S Aminoxy
92056	CF@405M Aminoxy
92051	CF@488A Aminoxy
92057	CF@568 Aminoxy
92052	CF@594 Aminoxy
92053	CF@633 Aminoxy
92058	CF@640R Aminoxy
92059	CF@660R Aminoxy
92054	CF@680R Aminoxy
92177	CF@488DI Aminoxy
92178	CF@555DI Aminoxy
92179	CF@647DI Aminoxy
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. Cy Dye and Sephadex are registered trademarks of GE Healthcare.

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