**Product Information**

**CF™ Dye Aminooxy**

**Unit Size:** 1 mg

**Technical Summary**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>CF™ Dye</th>
<th>Abs/Em maxima</th>
<th>MW</th>
<th>Extinction coefficient</th>
<th>Net charge*</th>
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<tbody>
<tr>
<td>92050</td>
<td>CF™350</td>
<td>347/448 nm</td>
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<td>92055</td>
<td>CF™405S</td>
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<td>CF™405M</td>
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<td>CF™488A</td>
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</tbody>
</table>

* At neutral pH, after conjugation

**Storage and Handling**

Store CF™ dye aminooxy at -20°C, protected from light. Stock solutions may be prepared in water, DMSO or DMF.

**Product Description**

CF™ dye, aminooxy are CF™ dyes with an aminooxy reactive group. Aminooxy groups react with molecules containing aldehyde or ketone groups to form a stable oxime bond. CF™ dyes are exceptionally bright, photostable, and water soluble. For more information, download the CF™ Dye Selection Guide at [www.biotium.com](http://www.biotium.com).

**General protocol for labeling protein aldehyde or protein ketone with CF dye aminooxy**

1. Prepare a 5 mM stock solution of CF aminooxy in water, DMSO or DMF.

2. For catalyst, we recommend to use aniline, 10X in acetate buffer (Biotium Cat#:91057) that is optimized for ligation reaction.

3. Prepare a stock solution of protein aldehyde or protein ketone in 1X PBS buffer, preferably reaching a concentration between 20 uM-100 uM. Low concentration may result in poor protein recovery yield or inefficient labeling.

For labeling glycoproteins, perform the steps in the Additional protocol for Glycoprotein Oxidation below.

4. Add 50 molar equivalents of CF aminooxy reagent to the solution prepared in step 3. 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 nmole, add 250 nmole of dye, which means 50 uL of 5 mM stock solution).

5. Initiate the ligation by adding 1/10 volume of aniline acetate catalyst (Cat#:91057). For example, if the mixture from step 4 is 150 uL in total, add 15 uL of catalyst.

6. Vortex the solution and allow the reaction to proceed at room temperature with agitation for 2 hr for protein aldehyde or 5 to 10 hr for protein ketone in the dark.

7. Purify the CF labeled protein conjugate by Sephadex G25 column or centrifugal protein concentrator. Biotium offers ultrafiltration vials with molecular weight cut-off of 10 kDa (Cat#:22004) or 3 kDa (Cat#:22018). 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.

8. 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 CF dye aminooxy, oxidation must be performed to convert glycoproteins to protein aldehydes before dye labeling.

1. Prepare 10X reaction buffer: 1 M sodium acetate; 1.5 M NaCl in DI water; pH5.5.

2. Prepare 100 mM sodium periodate (NaIO₄) 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 NaIO₄ 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 NaIO₄ 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. (The molarity of pure ethylene glycol is 14.5M, 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). Incubate 10 min, RT.

7. Proceed to the aminooxy labeling protocol step 4.

**Other Related Products**

Please visit [www.biotium.com](http://www.biotium.com) to view our full selection of CF™ reactive dyes, secondary antibody conjugates, and other conjugates, and many other innovative products for life science research.

CF dye technology is covered by granted U.S. and international patents.

Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.

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*www.biotium.com*