

# Product Information

## CF™ Dye Aminoxy

Unit Size: 1 mg

### Technical Summary

Cat. No.	CF™ Dye	Abs/Em maxima	MW	Extinction coefficient	Net charge*
92050	CF™350	347/448 nm	~567	18,000	-1
92055	CF™405S	404/431 nm	~642	33,000	-3
92056	CF™405M	408/452 nm	~574	41,000	-2
92051	CF™488A	490/515 nm	~1100	70,000	-2
92057	CF™568	562/583 nm	~786	100,000	-1
92052	CF™594	593/614 nm	~801	115,000	-1
92053	CF™633	630/650 nm	~893	100,000	-1
92058	CF™640R	642/662 nm	~1107	105,000	-2
92059	CF™660R	663/682 nm	~960	100,000	-1
92054	CF™680R	680/701 nm	~984	140,000	-1

\* At neutral pH, after conjugation

### Storage and Handling

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

### Product Description

CF™ dye, aminoxy are CF™ dyes with an aminoxy reactive group. Aminoxy 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 aminoxy

1. Prepare a 5 mM stock solution of CF aminoxy 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 aminoxy 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 aminoxy, 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 (NaIO<sub>4</sub>) 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<sub>4</sub> 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<sub>4</sub> 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 aminoxy 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.

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