Revised: September 9, 2024

# Product Information

## CF® Dye SE/TFP

See product page for a full list of product names, unit sizes, and catalog numbers.

### Storage and Handling

Store desiccated at ≤ -20°C. Product is stable for at least 1 year from date of receipt when stored as directed.

**Form:** Lyophilized solid

### Stock Solution Preparation:

Allow the vial of CF® Dye ester (SE or TFP) to warm up to room temperature. Prepare a 10 mM dye stock solution. For 1 umol dye: add 100 uL anhydrous DMSO to the vial. For 0.25 umol dye: add 25 uL anhydrous DMSO to the vial. Vortex the vial briefly to fully dissolve the dye, followed by brief centrifugation to collect the dye at the bottom of the vial. Unused stock solution may be stored at -20°C, protected from light and moisture. If anhydrous DMSO is used for making the solution, the dye should be stable for at least one month.

Dye stock solution may also be prepared in $dH_2O$ or aqueous buffer. However, because the dye will hydrolyze over time, aqueous stock solutions should be prepared immediately before the conjugation reaction and cannot be stored for later use.

### Product Description

Succinimidyl Ester (SE or NHS ester) CF® Dyes are amine-reactive forms of Biotium's bright and photostable CF® Dyes. The succinimidyl ester group of the dye reacts with an amine group to form a stable amide linkage. CF® Dyes are next-generation fluorescent dyes that have addressed the limitations of other commonly used fluorescent dyes for improved compatibility and signal-to-noise.

CF® Dye TFP (tetrafluorophenyl) esters are more stable alternatives to succinimidyl ester (SE or NHS ester). CF® Dye TFP esters are available for CF®850 and CF®870, Biotium's industry-leading near-infrared dyes with emission above 850 nm.

### Labeling Protocols

#### Considerations for protein labeling

The protocol provided below is a typical procedure for labeling IgG antibodies in bicarbonate buffer or IgM antibodies in PBS (pH~7.4). For labeling most other proteins or antibodies that are stable at pH 8.3, the IgG labeling protocol with the appropriate dye:protein ratio would be the most suitable. For IgG, 1 umol dye is sufficient to label 8-15 mg IgG; 0.25 umol dye is sufficient to label 2-3 mg IgG. The optimal dye amounts for labeling IgM or other proteins need to be determined empirically.

#### Considerations for nucleic acid labeling

CF® Dye Succinimidyl Esters can be used to label DNA modified with free amine groups, either by enzymatic incorporation of aminoallyl-modified nucleotides (see Related Products), or oligonucleotide synthesis with a terminal amino group. For oligonucleotide labeling, we recommend using an HPLC-purified oligo, with an amino group with a C6 linker at the 5' or 3' end. A method yielding the appropriate degree or purity should then be used to remove free dye after labeling, with common methods being ethanol precipitation, reverse phase HPLC, and cation exchange chromatography. Alternatively, an oligo synthesis company could perform this for you as a custom labeling.

### Antibody Labeling Protocol

#### Materials required but not provided

- IgG or IgM antibodies should be free of any amine-containing stabilizers, such as amino acids, Tris, BSA, or gelatin, as these substances will also react with the dye. Small molecules like Tris or amino acids can be removed by dialyzing the antibody against PBS buffer, or using an ultrafiltration vial to exchange the buffer (see Related Products). The presence of azide does not affect the labeling reaction.
- Anhydrous DMSO (see Related Products)
- Sodium bicarbonate ($NaHCO_3$)
- Sephadex®; view the product page for the appropriate type of Sephadex® media for each CF® Dye
- PBS buffer (pH~7.4)
- Sodium azide ($NaN_3$)
- Bovine Serum Albumin (BSA) (see Related Products)

## 1. Labeling procedure

1.1 Prepare antibody solution for labeling:

**For labeling IgG**

Dissolve the antibody in 0.1 M sodium bicarbonate buffer (pH~8.3) at 2.5 mg/mL. If the IgG is already dissolved in a buffer such as PBS, the labeling solution can be prepared by adding one-tenth volume of 1 M sodium bicarbonate solution (pH 8.3) to the IgG solution for a final bicarbonate concentration of 0.1 M.

**For labeling IgM**

Commonly used protocols for labeling IgM antibodies use buffer at neutral pH. Prepare your IgM antibody solution at 2.5 mg/mL to 5 mg/mL concentration in PBS (pH~7.4). Our Mix-n-Stain™ CF® Dye IgM Antibody Labeling Kits can also be used for fast and easy labeling of IgM (see Related Products).

**Note**: For IgG, the labeling efficiency is generally around 35% at 2.5 mg/mL protein concentration. A protein concentration of less than 2.5 mg/mL is also suitable for labeling, although labeling efficiency will be lower. A labeling efficiency of 20-30% can be expected with an IgG concentration around 1 mg/mL. Higher labeling efficiency is possible with an IgG concentration higher than 5 mg/mL. IgM labeling is much less efficient than IgG labeling because hydrolysis dominates the process at neutral pH. Because of variations in buffer and protein purity, accurate labeling efficiency can only be determined under your exact conditions. If the antibody solution is too dilute, it may be concentrated using an ultrafiltration vial with 10 kDa molecular weight cut-off (10 kDa MWCO; see Related Products).

1.2 Prepare dye stock solution:

See “Stock Solution Preparation” on page 1.

**Notes**:

a. For labeling IgM antibodies, you may need to prepare a more concentrated dye stock solution; see Section 1.3.

b. If the labeling reaction is to be carried out with a small amount of protein, the dye stock solution may need to be more dilute for accurate pipetting.

1.3 Carry out the labeling reaction:

**For labeling IgG**

While stirring or vortexing the protein solution, add 15-25 uL of the 10 mM dye stock per mL of antibody solution in a dropwise fashion. These volumes correspond to dye/IgG molar ratios between 9:1 to 15:1. Volume of dye added may need to be adjusted to achieve optimal DOL.

**For labeling IgM**

Labeling is less efficient for IgM antibodies because hydrolysis dominates over labeling at neutral pH. For this reason, the dye/IgM molar ratio needs to be on the order of 50:1 or 100:1. While stirring or vortexing the protein solution, add 70-140 uL of 10 mM dye stock per mL of antibody solution in a dropwise fashion. These values correspond to dye/IgM molar ratios between 50:1 to 100:1. The concentration of dye in the stock solution may be increased up to 20 mM if more dye is needed to achieve an optimal DOL.

**Note**: If IgM labeling efficiency is poor, an overnight incubation at 4°C with a 30:1 dye/IgM molar ratio may reduce hydrolysis and improve labeling efficiency.

1.4 Continue to stir or rock the reaction solution at room temperature for 1 hour, protected from light.

**Note**: While the labeling reaction is underway, proceed to Step 1.5a to prepare a Sephadex® column. View the product page for the appropriate Sephadex® medium to use for each CF® Dye. For small-scale labeling reactions, you may use an ultrafiltration vial (see Related Products) to remove the free dye from the conjugate in order to avoid an overly dilute product. 10K MWCO can be used for IgG or IgM; proteins with different molecular weights may require different MWCO. If you choose not to separate the labeled antibody from the free dye immediately after the reaction, you may add 50 uL of 1 M lysine to stop the reaction.

1.5 Separate the labeled protein from the free dye:

a. Prepare a Sephadex® column (10 mm x 300 mm) equilibrated in PBS buffer (pH~7.4).

b. After incubation, load the reaction solution from Step 1.3 onto the column and elute the column with PBS buffer. The first band excluded from the column corresponds to the antibody conjugate.

c. Proceed to determination of degree of labeling (next page).

## 2. Determination of degree of labeling (DOL)

2.1 Determine the protein concentration:

The concentration of the antibody conjugate can be calculated from the formula:

$$[\text{conjugate}] = \{[A_{280} - (A_{max} \times C_f)]/\varepsilon_{prot}\} \times \text{dilution factor}$$

Where [conjugate] is the concentration of the antibody conjugate collected from the column in mg/mL; "dilution factor" is the fold of dilution used for spectral measurement; $A_{280}$ and $A_{max}$ are the absorbance readings of the conjugate at 280 nm and the absorption maximum respectively; $C_f$ is the absorbance correction factor; and the value $\varepsilon_{prot}$ is the extinction coefficient in mL/mg. The extinction coefficients for IgG and IgM are 1.4 and 1.18 respectively. View the product page for the $A_{max}$ and correction factor for each CF® Dye.

**Note**: The protein solution eluted from the column may be too concentrated for accurate absorbance measurement and thus must be diluted to approximately ~0.1 mg/mL. The fold of dilution ("dilution factor") necessary can be estimated from the amount of starting antibody (i.e., 5 mg) and the total volume of the protein solution collected from the column.

2.2 Calculate the degree of labeling (DOL):

The DOL is calculated according to the formula:

$$\text{DOL} = (A_{max} \times \text{Mwt} \times \text{dilution factor})/(\varepsilon_{dye} \times [\text{conjugate}])$$

Where $A_{max}$, "dilution factor" and [conjugate] are as defined in Step 2.1, Mwt is the molecular weight of IgG (~150,000) or IgM (~180,000), and $\varepsilon_{dye}$ is the molar extinction coefficient of the dye (see product page). The product page lists the optimal range of DOL for each dye, although a DOL slightly above or below this range will also produce good results. If labeling a protein other than immunoglobulin, use the extinction coefficient for that specific protein.

## 3. Storage and handling of labeled antibody

For long-term storage, we recommend adding 5-10 mg/mL BSA and 0.01-0.03% sodium azide to the conjugate solution to prevent denaturation and microbial growth. The conjugate solution should be stored at 4°C and protected from light. If glycerol is added to a final concentration of 50%, the conjugate can be stored at -20°C. Under these conditions, antibody conjugates are stable for a year or longer.

## Related Products

| Cat. No. | Product |
|---|---|
| 92210-92226 | CF® Dye & Biotin SE Protein Labeling Kits |
| 92020...96079 | CF® Dye Maleimides |
| 92096-92099 | CF® Dye MTS |
| 92050-92059 | CF® Dye Aminooxy |
| 92151...96064 | CF® Dye Hydrazides |
| 92035-92102 | CF® Dye Amine |
| 92080..96000 | CF® Dye Azide |
| 92086...96006 | CF® Dye Alkyne |
| 92230...92433 | Mix-n-Stain™ CF® Dye Antibody Labeling Kits |
| 92558...92575 | Mix-n-Stain™ CF® Dye IgM Antibody Labeling Kits |
| 40020 | 5-Aminoallyl-dUTP |
| 40021 | 5-Aminoallyl-UTP |
| 22004 | Ultrafiltration Vial, 10K MWCO (5 per pack) |
| 22018 | Ultrafiltration Vial, 3K MWCO (5 per pack) |
| 90082 | DMSO, Anhydrous |
| 22013 | Bovine Serum Albumin, Fraction V |
| 22014 | Bovine Serum Albumin, 30% Solution |
| 22023 | Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative |
| 22030 | AntiFix™ Universal Antigen Retrieval Buffer, 10X |
| 22010 | 10X Fish Gelatin Blocking Agent |
| 23002 | EverBrite™ Mounting Medium with DAPI |
| 23004 | EverBrite™ Hardset Mounting Medium with DAPI |
| 40060 | RedDot™1 Far-Red Nuclear Stain |
| 40061 | RedDot™2 Far-Red Nuclear Stain |
| 40083...41038 | NucSpot® Nuclear Stains for Dead or Fixed Cells |
| 40081, 40082 | NucSpot® Live Nuclear Stains |
| 41024-4L | Water, Ultrapure Molecular Biology Grade |

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF® Dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.