

# Product Information

## CF™ 568-dUTP

**Catalog Number** 40005

**Unit Size** 25 nmol

**Molecular Weight** ~1476

**Color and Form** Red solid

### Spectral Properties

$\lambda_{\text{abs}}/\lambda_{\text{em}} = 562/583$  nm (Figure 1)

Extinction coefficient: 100,000

CF™568 is spectrally similar to Alexa Fluor® 568, ATTO™ 565, and Rhodamine Red

### Storage and Handling

Store desiccated at  $\leq -20^{\circ}\text{C}$ . When stored as recommended, product is stable for at least 6 months from date of receipt. For aqueous solutions, prepare single use aliquots and store protected from light at  $-20^{\circ}\text{C}$  for up to 6 months. We recommend preparing a 1 mM stock solution in 10 mM Tris pH 7.4.

### Product Application

CF™568 is a red fluorescent dye with an excitation spectrum optimally matching the 568 nm line of the Ar-Kr mixed-gas laser (Figure 1). The photostability of CF™568 is superior to that of Alexa Fluor® 568, making CF™568 a better choice for demanding applications such as confocal microscopy and single molecule imaging.

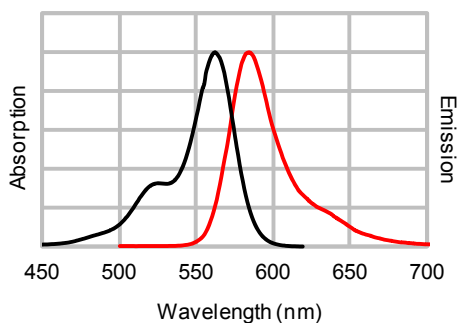
CF™-568 dUTP can be used for TUNEL staining<sup>1</sup>, or can be used in place of dTTP in standard DNA labeling and synthesis protocols to generate fluorescent dsDNA and oligonucleotide probes.

Note: for PCR applications, Taq polymerase should be used with dUTP conjugates, because dUTP inhibits archaeal polymerases such as *Pfu* and Vent.<sup>2,3</sup>

### References

1. Gold et al. (1994). Lab Invest. 71 (2):219-25.
2. Slupphaug et al. (1993). Anal Biochem. 211 (1):164-9.
3. Hogrefe et al. (2002). PNAS 99 (2): 596-601.

Figure 1. Absorption/Emission Spectra of CF™568 Conjugates



## General protocol for terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) of apoptotic cells

### 1. Materials Required but not Provided

- Phosphate buffered saline pH 7.4 (PBS)
- 4% formaldehyde/PBS
- 70% ethanol (optional)
- PBS/0.2% TX-100
- PBS/0.1% TX-100/5 mg/mL bovine serum albumin (BSA)
- 12.5 U/ $\mu\text{L}$  recombinant terminal transferase (TdT) enzyme
- 5X TdT reaction buffer: 1M potassium cacodylate, 125 mM Tris-HCl, 1.25 mg/mL BSA, pH 6.6
- 25 mM  $\text{CoCl}_2$  solution
- 100  $\mu\text{M}$  dATP

### 2. Sample preparation

- 2.1 Preparation of cells or fresh-frozen tissue sections
  - a) Optional: include an extra sample to perform a negative control TUNEL reaction without TdT enzyme.
  - b) Wash cells or sections twice in PBS.
  - c) Fix cells or tissues in 4% formaldehyde in PBS (pH 7.4) for 30 minutes at  $4^{\circ}\text{C}$ .
  - e) Optional: store cells in 70% ethanol at  $-20^{\circ}\text{C}$  for up to two weeks, proceed to (f).
  - d) Wash twice in PBS.
  - e) Permeabilize in 0.2% TX-100 in PBS for 30 minutes at room temperature.
  - f) Wash twice in PBS.
- 2.2 Preparation of paraffin tissue sections
  - a) Optional: include an extra sample to perform negative control (no TdT enzyme) TUNEL labeling.
  - b) Deparaffinize and rehydrate sections according to standard protocols.
  - c) Wash twice in PBS.
  - d) Permeabilize sections with 20  $\mu\text{g}/\text{mL}$  proteinase K in PBS for 30 minutes at room  $37^{\circ}\text{C}$ . Proteinase K incubation time and temperature may require optimization depending on tissue type. Alternatively, microwave antigen retrieval protocols may be used at this step.
  - e) Wash several times in PBS.

### 3. Reaction mix preparation

- 3.1 Prepare a 10  $\mu\text{M}$  stock solution stock of CF™ dye-dUTP in  $\text{dH}_2\text{O}$ .
- 3.2 Prepare 100  $\mu\text{L}$  of TUNEL equilibration buffer per sample according to Table 1.
- 3.3 Prepare 50  $\mu\text{L}$  of TUNEL reaction mix per sample according to Table 1.
  - a) Optional: prepare negative control reaction mix without TdT enzyme according to Table 1.

Continued next page

**Table 1. Preparation of TUNEL equilibration and reaction buffers**

Component	Volume per reaction ( $\mu\text{L}$ )			
	Equilibration buffer	Reaction mix	No TdT control	Final concentration
5X TdT reaction buffer	20	10	10	1X
25 mM $\text{CoCl}_2$	20	10	10	5 mM
100 $\mu\text{M}$ dATP	-	2.5	2.5	5 $\mu\text{M}$
10 $\mu\text{M}$ CF <sup>TM</sup> dye-dUTP	-	2.5	2.5	0.5 $\mu\text{M}$
12.5 U/ $\mu\text{L}$ TdT	-	1	-	12.5 U/reaction
$\text{dH}_2\text{O}$	60	24	25	
Final volume ( $\mu\text{L}$ )	100	50	50	

#### 4. TUNEL staining

4.1 Incubate samples with 100  $\mu\text{L}$  equilibration buffer for 5 minutes at room temperature.

a) For adherent cells or tissue sections, cover sample with a Parafilm coverslip to spread buffer evenly over the cells or tissue section.

4.2 Remove equilibration buffer and add 50  $\mu\text{L}$  of reaction buffer to each sample.

a) For adherent cells or tissue sections, cover sample with a Parafilm coverslip to spread buffer evenly over cells or tissue section.

4.3 Incubate samples for 60 minutes at 37°C, protected from light. Tissue sections may require 2 hour incubation at 37°C.

a) For adherent cells or tissue sections, perform incubation in a humid chamber.

b) For cells in suspension, perform incubation in a microplate on a rocking platform, or resuspend cells in reaction buffer every 15 minutes by gently flicking tubes.

4.4 Wash samples twice in PBS/0.1% TX-100/5 mg/mL BSA.

4.5 Counterstain samples if desired. Mount samples in fluorescence mounting medium and coverslip for microscopy, or analyze cells in suspension by flow cytometry.

#### Related Products

Cat. #	Product description
40004	CF <sup>TM</sup> 405S-dUTP, 25 nmol
40008	CF <sup>TM</sup> 488A-dUTP, 25 nmol
40002	CF <sup>TM</sup> 543-dUTP, 25 nmol
40006	CF <sup>TM</sup> 594-dUTP, 25 nmol
40007	CF <sup>TM</sup> 640R-dUTP, 25 nmol
40003	CF <sup>TM</sup> 680R-dUTP, 25 nmol
40027	CF <sup>TM</sup> 555-dCTP, 25 nmol
40028	CF <sup>TM</sup> 647-dCTP, 25 nmol
30063	CF <sup>TM</sup> 488A TUNEL Assay Apoptosis Detection Kit, 50 reactions
30064	CF <sup>TM</sup> 594 TUNEL Assay Apoptosis Detection Kit, 50 reactions

Please visit our website at [www.biotium.com](http://www.biotium.com) to view our full selection of CF<sup>TM</sup> dye bioconjugates, including antibodies, antibody labeling kits, phalloidin, annexin V and  $\alpha$ -bungarotoxin, as well as fluorescent reagents and kits for genomics and cell biology research.

CF dye technology is covered by pending US and international patents. Alexa Fluor is a registered trademarks of Invitrogen; DyLight is a registered trademark of Thermo Fisher Scientific.

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