

Product Information

CF™568-dUTP

Catalog Number 40005

Unit Size 25 nmol

Technical Summary

Abs/Em Maxima: 562/583 nm (See Figure 1)

Extinction coefficient: 100,000

Molecular weight: ~1476

Direct replacement for: dUTP conjugated to Alexa Fluor® 568, ATTO™ 565, DY-560, Rhodamine Red

Color and Form

Dark red solid

Storage and Handling

Store CF™568-dUTP desiccated at $\leq -20^{\circ}\text{C}$. When stored as directed, CF™568-dUTP should be stable for at least 6 months from the time of receipt. For aqueous solutions of CF™568-dUTP, prepare single use aliquots and store protected from light at -20°C for up to 6 months. Avoid freeze-thaw cycles.

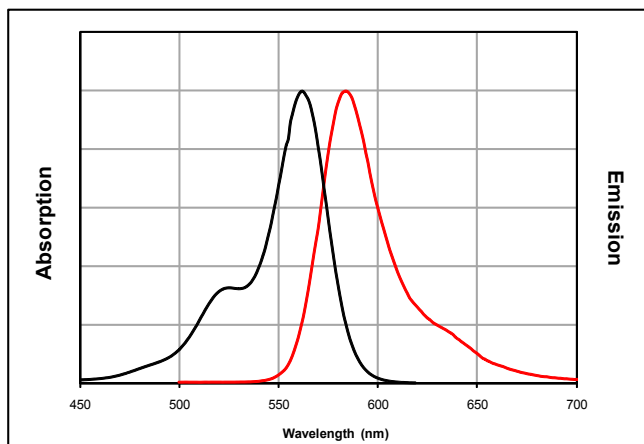
Solubility

Soluble in H_2O . We recommend preparing a 1 mM stock solution in dH_2O .

Spectral Properties

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). Antibody conjugates of the dye are much brighter than those of Alexa Fluor® 568. In addition, the photostability of CF™568 is superior to that of Alexa Fluor® 568, making CF™568 a much better choice for demanding applications, such as confocal and single molecular imaging. Fluorophore conjugates of dUTP can be used for TUNEL staining, or can be used in place of dTTP in standard DNA labeling and synthesis protocols to generate fluorescent dsDNA and oligonucleotide probes.

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



Product Application

CF™568-dUTP can be used for detection of apoptotic cells by direct fluorescence TUNEL labeling of DNA strand breaks. Fluorophore conjugates of dUTP can be used in place of dTTP in standard DNA labeling and synthesis protocols to generate fluorescent dsDNA and oligonucleotide probes.

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/ μL 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 CoCl_2 solution
- 100 μM dATP

2. Sample preparation

2.1 Preparation of cells or fresh-frozen tissue sections

- Optional: include an extra sample to perform a negative control TUNEL reaction without TdT enzyme.
- Wash cells or sections twice in PBS.
- Fix cells or tissues in 4% formaldehyde in PBS (pH 7.4) for 30 minutes at 4°C .
- Optional: store cells in 70% ethanol at -20°C for up to two weeks, proceed to (f).
- Wash twice in PBS.
- Permeabilize in 0.2% TX-100 in PBS for 30 minutes at room temperature.
- Wash twice in PBS.

2.2 Preparation of paraffin tissue sections

- Optional: include an extra sample to perform negative control (no TdT enzyme) TUNEL labeling.
- Deparaffinize and rehydrate sections according to standard protocols.
- Wash twice in PBS.
- Permeabilize sections with 20 $\mu\text{g}/\text{mL}$ proteinase K in PBS for 30 minutes at room 37°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.
- Wash several times in PBS.

Continued on page 2

3. Reaction mix preparation

3.1 Prepare a 10 μ M stock solution of CF[™]568-dUTP in dH₂O.

3.2 Prepare 100 μ L of TUNEL equilibration buffer per sample according to Table 1.

3.3 Prepare 50 μ 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.

Table 1. Preparation of TUNEL equilibration and reaction buffers

Component	Volume per reaction (μ L)			
	Equilibration buffer	Reaction mix	No TdT control	Final concentration
5X TdT reaction buffer	20	10	10	1X
25 mM CoCl ₂	20	10	10	5 mM
100 μ M dATP	-	2.5	2.5	5 μ M
10 μ M CF [™] 568-dUTP	-	2.5	2.5	0.5 μ M
12.5 U/ μ L TdT	-	1	-	12.5 U/reaction
dH ₂ O	60	24	25	
Final volume (μ L)	100	50	50	

4. TUNEL staining

4.1 Incubate samples with 100 μ 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 μ 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

Catalog number	Product	Description
30063	CF [™] 488A TUNEL Assay Apoptosis Detection Kit	Kit contains equilibration buffer, reaction buffer, and TdT enzyme for CF [™] 488A-dUTP TUNEL staining.
30064	CF [™] 594 TUNEL Assay Apoptosis Detection Kit	Kit contains equilibration buffer, reaction buffer, and TdT enzyme for CF [™] 594-dUTP TUNEL staining.

Additional CF[™] dye dUTP conjugates

Catalog number	Product description	Abs/Em Maxima (nm)	Direct replacement for dUTP conjugated to:
40004	CF [™] 405S-dUTP	404/431	Cascade® Blue, DyLight® 405
40008	CF [™] 488A-dUTP	490/515	Alexa Fluor® 488, DyLight® 488, Fluorescein, FITC, Cy [™] 2
40006	CF [™] 594-dUTP	593/614	Alexa Fluor® 594, DyLight® 594, Texas Red
40007	CF [™] 640R-dUTP	642/662	Alexa Fluor® 647, Cy [™] 5

CF[™] dye technology is covered by pending US and international patents. Alexa Fluor® and Cascade Blue® are registered trademarks of Invitrogen; Cy[™] is a trademark of GE Healthcare; 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. Biotium is not liable for any damage resulting from handling or contact with this product.