

# Product Information

## CF™488A-dUTP

**Catalog Number** 40008

**Unit Size** 25 nmol

### Technical Summary

**Abs/Em Maxima:** 490/515 nm (See Figure 1)

**Extinction coefficient:** 70,000

**Molecular weight:** ~1429

**Direct replacement for:** dUTP conjugated to fluorescein, FITC  
Alexa Fluor®488, Cy™2 or DyLight®488

### Color and Form

Yellow solid

### Storage and Handling

Store CF™488A-dUTP desiccated at  $\leq -20^{\circ}\text{C}$ . When stored as directed, CF™488A-dUTP should be stable for at least 6 months from the time of receipt. For aqueous solutions of CF™488A-dUTP, prepare single use aliquots and store protected from light at  $-20^{\circ}\text{C}$  for up to 6 months. Avoid freeze-thaw cycles.

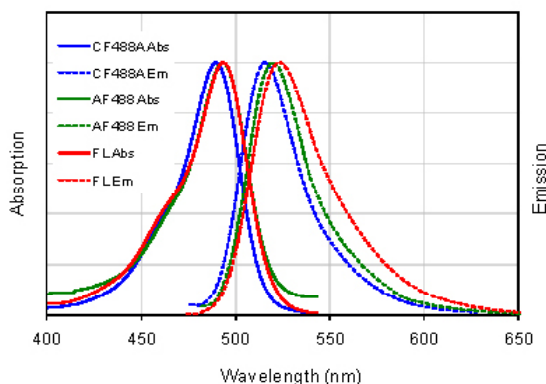
### Solubility

Soluble in  $\text{H}_2\text{O}$ . We recommend preparing a 1 mM stock solution in  $\text{dH}_2\text{O}$ .

### Spectral Properties

CF™488A is a green fluorescent dye optimally excitable by the 488 nm argon laser line. Under common detection conditions, CF™488A is at least as bright as Alexa Fluor® 488. In addition, the emission peak wavelength of CF™488A is about 10 nm shorter than that of Alexa Fluor® 488 and 15 nm shorter than that of the traditional green dye FITC (or FAM) (see Figure 1). The shorter wavelength of CF™488A offers the advantage of less fluorescence "spill-over" in the red channel in multicolor detection applications.

Figure 1. Absorption/emission spectra of CF™488A, Alexa Fluor® 488 (AF488) and FITC (FL) conjugates.



## Product Application

CF™488A-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/ $\mu\text{L}$  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

- 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  $4^{\circ}\text{C}$ .
- Optional: store cells in 70% ethanol at  $-20^{\circ}\text{C}$  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  $37^{\circ}\text{C}$ . Proteinase K incubation time and temperature may require optimization depending on tissue type. Alternatively, microwave antigen retrieval protocols may be used at this step.
- Wash several times in PBS.

Continued on page 2

### 3. Reaction mix preparation

3.1 Prepare a 10  $\mu$ M stock solution of CF™488A-dUTP in dH<sub>2</sub>O.

3.2 Prepare 100  $\mu$ L of TUNEL equilibration buffer per sample according to Table 1.

3.3 Prepare 50  $\mu$ 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.

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.

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

Component	Volume per reaction ( $\mu$ L)			
	Equilibration buffer	Reaction mix	No TdT control	Final concentration
5X TdT reaction buffer	20	10	10	1X
25 mM CoCl <sub>2</sub>	20	10	10	5 mM
100 $\mu$ M dATP	-	2.5	2.5	5 $\mu$ M
10 $\mu$ M CF™488A-dUTP	-	2.5	2.5	0.5 $\mu$ M
12.5 U/ $\mu$ L TdT	-	1	-	12.5 U/reaction
dH <sub>2</sub> O	60	24	25	
Final volume ( $\mu$ L)	100	50	50	

### 4. TUNEL staining

4.1 Incubate samples with 100  $\mu$ 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  $\mu$ 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
40005	CF™568-dUTP	562/583	Alexa Fluor® 568, Rhodamine Red
40006	CF™594-dUTP	593/614	Alexa Fluor® 594, DyLight® 594, Texas Red
40007	CF™640R-dUTP	642/662	Alexa Fluor® 647, Cy™5