Revised: April 25, 2011

Product Information

CF™405S-dUTP

Catalog Number 40004

Unit Size 25 nmol

Technical Summary

Abs/Em Maxima: 404/431 nm (See Figure 1)

Extinction coefficient: 33,000

Molecular weight: ~1684

Direct replacement for: dUTP conjugated to Cascade Blue®, DyLight® 405

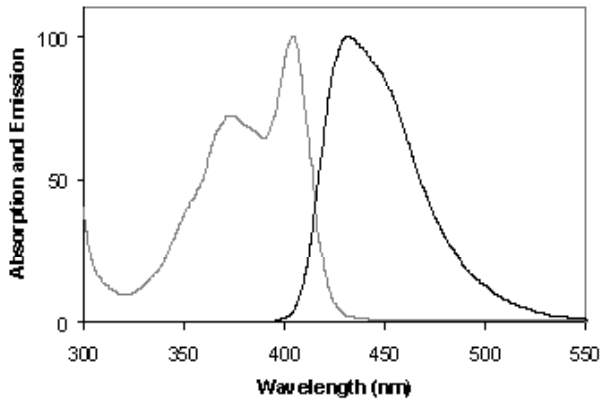
Color and Form
Colorless solid

Storage and Handling
Store CF™405S-dUTP desiccated at ≤ -20°C. When stored as directed, CF™405S-dUTP should be stable for at least 6 months from the time of receipt. For aqueous solutions of CF™405S-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™405S is a blue fluorescent dye with an absorption peak wavelength that nearly coincides with the 405 nm blue diode laser line (Figure 1). In addition, the emission peak wavelength of the dye well centers within the blue detection window of BD flow cytometers.

Figure 1. Absorption/emission spectra for CF™405S conjugates



Product Application

CF™405S-dUTP can be used for detection of apoptotic cells by direct fluorescence TUNEL labeling of DNA strand breaks in cells. **Please note:** CF™405S-dUTP may not be suitable for TUNEL staining in tissues due to blue autofluorescence in tissues and lower incorporation efficiency compared to other CF™dye dUTP conjugates. 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.

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/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

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 4ºC.

e) Optional: store cells in 70% ethanol at -20ºC 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 μg/mL proteinase K in PBS for 30 minutes at room 37ºC. 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.

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3. Reaction mix preparation

3.1 Prepare a 10 μM stock solution stock of CF™dye-dUTP in dH_2O.

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

3.3 Prepare 50 μL 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 (μL)			
	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 μM dATP	-	2.5	2.5	5 μM
10 μM CF™dye-dUTP	-	2.5	2.5	0.5 μM
12.5 U/μL TdT	-	1	-	12.5 U/reaction
dH_2O	60	24	25	
Final volume (μL)	100	50	50	

4. TUNEL staining

4.1 Incubate samples with 100 μL 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 μL 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 37ºC, protected from light. Tissue sections may require 2 hour incubation at 37ºC.

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	Abs/Em Maxima (nm)	Direct replacement for dUTP conjugated to:
40008	CF™488A-dUTP	490/515	Alexa Fluor® 488, DyLight® 488, Fluorescein, FITC, Cy™2
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

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.

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