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Product Information

CF™640R-dUTP

Catalog Number 40007

Unit Size 25 nmol

Molecular Weight ~1594

Color and Form Blue solid

Spectral Properties
$\lambda_{abs}/\lambda_{em}$ = 642/662 nm (Figure 1)

Extinction coefficient: 105,000

CF™640R is spectrally similar to Alexa Fluor® 647, ATTO 647N, Cy®5, and DyLight® 649

Storage and Handling
Store desiccated at ≤ -20°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°C for up to 6 months. We recommend preparing a 1 mM stock solution in 10 mM Tris pH 7.4.

Product Application

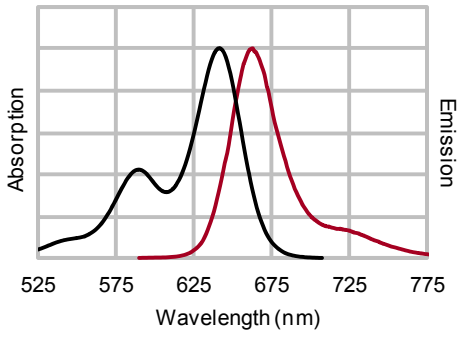
Far-red CF™640R is much brighter than Cy™5 and at least as bright as Alexa Fluor® 647. A major advantage of CF™640R over Cy™ 5 and Alexa Fluor® 647 is its exceptional photostability. CF™640R is also superior to ATTO 647N, another spectrally similar dye frequently used in single-molecule imaging. The combination of excellent brightness and photostability makes CF™640R ideal for confocal microscopy, single- molecule imaging and other demanding applications based on fluorescence detection.

Fluorophore conjugates of dUTP can be used for TUNEL staining[1], 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.[2,3]

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 and emission spectra of CF™640R 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/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.

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.

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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

Cat. #	Product description
40004	CF™405S-dUTP, 25 nmol
40008	CF™488A-dUTP, 25 nmol
40002	CF™543-dUTP, 25 nmol
40005	CF™568-dUTP, 25 nmol
40006	CF™594-dUTP, 25 nmol
40003	CF™680R-dUTP, 25 nmol
40027	CF™555-dCTP, 25 nmol
40028	CF™647-dCTP, 25 nmol
30063	CF™488A TUNEL Assay Apoptosis Detection Kit, 50 reactions
30064	CF™594 TUNEL Assay Apoptosis Detection Kit, 50 reactions

Please visit our website at **www.biotium.com** to view our full selection of CF™ dye bioconjugates, including antibodies, antibody labeling kits, phalloidin, annexin V and α-bungarotoxin, as well as fluorescent reagents and kits for genomics and cell biology research.