Revised: November 1, 2016

Product Information

CF™ Dye dUTP Conjugates

Catalog number	Size	Product	Molecular weight	Ex/Em (nm)
40004-T	5 nmol	CF™405S-dUTP	~1684	404/431
40004	25 nmol			
40008-T	5 nmol	CF™488A-dUTP	~1429	490/515
40008	25 nmol			
40002-T	5 nmol	CF™543-dUTP	~1385	541/560
40002	25 nmol			
40005-T	5 nmol	CF™568-dUTP	~1476	562/583
40005	25 nmol			
40006-T	5 nmol	CF™594-dUTP	~1491	593/614
40006	25 nmol			
40007-T	5 nmol	CF™640R-dUTP	~1594	642/662
40007	25 nmol			
40003-T	5 nmol	CF™680R-dUTP	~1675	680/701
40003	25 nmol			

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. Avoid freeze-thaw cycles. We recommend preparing a 1 mM stock solution in 10 mM Tris pH 7.4.

Product Application

CF™ dyes are Biotium's next-generation fluorescent dyes, with combined advantages in brightness, photostability, and water solubility compared to other dyes like Alexa Fluor®, DyLight®, Cy® Dye, and IRDye®. CF dye 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: CF™405S-dUTP may not be suitable for TUNEL staining in tissues due to blue autofluorescence in tissues and lower incorporation efficiency in tissue sections compared to other CF™dye dUTP conjugates.

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.

Protocols

DNA labeling by PCR

1. Materials Required but not Provided

- Taq DNA polymerase (see note under product application)
- 10X Taq reaction buffer
- 25 mM $MgCl_2$
- dATP, dTTP, dCTP, dGTP (separate solutions), 1 mM each
- DNA template
- Forward and reverse primers, 10 uM each
- PCR clean-up kit (optional)

2. PCR reaction

2.1 For each labeling reaction, set up the PCR reaction mix as shown below:

Component	Volume per reaction	Final concentration (after addition of dUTP)
10X Taq reaction buffer	2 uL	1X
25 mM $MgCl_2$	2 uL	5 mM
1 mM dATP	2 uL	100 uM
1 mM dCTP	2 uL	100 uM
1 mM dGTP	2 uL	100 uM
1 mM dTTP	1 uL	50 uM
10 uM forward primer	1 uL	500 nM
10 uM reverse primer	1 uL	500 nM
Template	1 ng	50 pg/uL
Taq	1 U	0.05 U/uL
Molecular grade dH_20	to 19 uL total	

2.2 Add 1 uL of 1 mM CF dye dUTP to the reaction tube.
Optional: for an unlabeled control reaction, add 1 uL of 1 mM dTTP instead of CF dye dUTP.

2.3 Perform PCR according to the following cycling protocol:

Denaturing/hot-start Taq activation 94°C, 2 min. (see note 1)	Hold
Denaturing 94°C 30 sec.	Cycle 30X
Annealing (see note 2) 30 sec.	
Extension 72°C 1 min. (see note 3)	
Final extension 72°C 5 min.	Hold

Notes:

1. This protocol was optimized for Cheetah™ Hot Start Taq polymerase (see related products). Other hot-start Taq polymerases may require longer activation times.
2. Set the annealing temperature 5°C below the melting temperature (T_m) of your primers.
3. This cycling protocol was optimized for 200-300 bp amplicons. Longer amplicons may require longer extension times.

2.4 Optional: use a PCR clean-up kit to remove unincorporated nucleotides.

2.5 Run 10% of the labeled product on an agarose gel without DNA dye added to analyze the efficiency and specificity of the PCR reaction. CF dye fluorescence can be imaged on a UV light box or laser-based gel scanner. Note: Far-red fluorescence emission (650 nm or longer) is not visible to the human eye.

2.6 Post-stain the gel with DNA gel stain to image the total PCR product or optional unlabeled control PCR product.

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Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) of apoptotic cells

Note: Biotium also offers CF Dye TUNEL Assay Kits with a selection of dye colors, which include Equilibration Buffer, CF dye TUNEL reaction buffer, and TdT enzyme (see related products).

1. Materials Required but not Provided

- Phosphate buffered saline pH 7.4 (PBS)
- 4% formaldehyde/PBS
- 70% ethanol (optional)
- PBS/0.2% Triton™ X-100
- PBS/0.1% Triton™ X-100/5 mg/mL bovine serum albumin (BSA)
- 12.5 U/uL 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 samples in 4% formaldehyde in PBS for 30 minutes at 4ºC.
e) Optional: store cells in 70% ethanol at -20ºC for up to two weeks
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 Dilute CF dye-dUTP to 10 uM in dH_2O.

3.2 Prepare 100 uL of TUNEL equilibration buffer per sample:
20 uL 5X TdT reaction buffer
20 uL 25 mM $CoCl_2$
60 uL dH_2O

3.3 Prepare 50 uL of CF dye TUNEL reaction mix for each sample:

TUNEL Reaction Mix

Component	Volume per reaction	Final concentration
5X TdT reaction buffer	10 uL	1X
25 mM $CoCl_2$	10 uL	5 mM
100 uM dATP	2.5 uL	5 uM
10 uM CF dye-dUTP	2.5 uL	0.5 uM
12.5 U/uL TdT	1 uL	12.5 U/reaction
dH_2O	24 uL	
Final volume	50 uL	

Optional: prepare a negative control sample without TdT enzyme.

4. TUNEL staining

4.1 Incubate samples with 100 uL 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 uL of reaction buffer to each sample.

a) For adherent cells or tissue sections, cover sample with a Parafilm® coverslip to spread buffer evenly over the 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 3 x 5 minutes in PBS/0.1% Triton X-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. TUNEL-positive cells should show bright nuclear fluorescence. No staining should be observed in the absence of TdT enzyme.

Related Products

Catalog No.	Product
30063	CF™488A TUNEL Assay Apoptosis Detection Kit
30064	CF™594 TUNEL Assay Apoptosis Detection Kit
30074	CF™640R TUNEL Assay Apoptosis Detection Kit
40067	CF™488A-dCTP
40057	CF™532-dCTP
40058	CF™543-dCTP
40027	CF™555 dCTP
40055	CF™568-dCTP
40056	CF™594-dCTP
40066	CF™640R-dCTP
40028	CF™647 dCTP
40068	CF™660R-dCTP
40031	CF™555 ddCTP
40032	CF640R UTP
40001	5-Tetramethylrhodamine-dUTP
40063	Fluorescein-12-dUTP
40059	DEAC-dUTP
40029	Biotin-11-dUTP
40022	Biotin-16-dUTP
40030	Biotin-20-dUTP
40035	Biotin-11-CTP
40036	Biotin-11-dCTP
40033	Biotin-11-UTP
40023	Biotin-16-UTP
40034	Biotin-20-UTP
40078	Digoxigenin-dUTP, alkali stable
40020	5-Aminoallyl-dUTP
40021	5-Aminoallyl-UTP
40052	dNTP Set, 100 mM each
29050	Cheetah™ Hot Start Taq DNA Polymerase
41003	GelRed™ Nucleic Acid Gel Stain, 10,000X in water
41004	GelGreen™ Nucleic Acid Gel Stain, 10,000X in water

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.

CF dye technology is covered by pending US and international patents.

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