Revised: February 11, 2021

Product Information

CF® Dye dCTP Conjugates

Catalog no.	Dye/Product	Unit size	Ex/Em (nm)	MW (free acid form)
40067-T	CF®488A-dCTP	5 nmol	490/515	~1428
40067		25 nmol		
40057-T	CF®532-dCTP	5 nmol	527/558	~1624
40057		25 nmol		
40058-T	CF®543-dCTP	5 nmol	541/560	~1647
40058		25 nmol		
40027-T	CF®555-dCTP	5 nmol	555/565	~1553
40027		25 nmol		
40055-T	CF®568-dCTP	5 nmol	562/583	~1652
40055		25 nmol		
40056-T	CF®594-dCTP	5 nmol	593/614	~1667
40056		25 nmol		
40066-T	CF®640R-dCTP	5 nmol	642/662	~1593
40066		25 nmol		
40028-T	CF®647-dCTP	5 nmol	650/665	~1579
40028		25 nmol		
40068-T	CF®660R-dCTP	5 nmol	663/682	~1826
40068		25 nmol		

Storage and Handling

Store desiccated at ≤ -20°C, protected from light. Product is stable for at least 6 months from date of receipt when stored as recommended.

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 Description

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 conjugates of dCTP can be used in standard DNA labeling and synthesis protocols to generate fluorescent dsDNA and oligonucleotide probes.

Note: CF®594 dCTP is not compatible with PCR labeling of DNA probes.

DNA Labeling by PCR

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)

PCR Reaction

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	1 uL	50 uM
1 mM dGTP	2 uL	100 uM
1 mM dTTP	2 uL	100 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. Add 1 uL of 1 mM CF® dye dCTP to the reaction tube and mix well.

   Optional: For an unlabeled control reaction, add 1 uL of 1 mM dTCP (unlabeled) instead of CF® dye dCTP.

3. Perform PCR according to the following cycling protocol:

Denaturing/hot-start Taq activation 94°C, 2 min.[a]	Hold
Denaturing 94°C 30 sec.	Cycle 30X
Annealing 30 sec.[b]	
Extension 72°C 1 min.[c]	
Final extension 72°C 5 min.	Hold

Notes:

a. This protocol was optimized for Cheetah™ HotStart Taq Polymerase (see Related Products). Other hot-start Taq polymerases may require longer activation times.

b. Set the annealing temperature 5°C below the melting temperature (T_m) of your primers.

c. This cycling protocol was optimized for 200-300 bp amplicons. Longer amplicons may require longer extension times.

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

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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

   Notes:

   a. Far-red fluorescence emission (650 nm or longer) is not visible to the human eye.

   b. Be sure to image CF® dye fluorescence before staining DNA with gel stain, because CF® dyes and gel stains may quench one another.

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

Related Products

Catalog number	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
40004	CF®405S-dUTP
40008	CF®488A-dUTP
40002	CF®543-dUTP
40005	CF®568-dUTP
40006	CF®594-dUTP
40007	CF®640R-dUTP
40003	CF®680R-dUTP
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™ HotStart Taq DNA Polymerase
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in water
41004	GelGreen® Nucleic Acid Gel Stain, 10,000X in water
41029	GelRed® Agarose LE
41030	GelGreen® Agarose LE
41028	Agarose LE, Ultra-Pure Molecular Biology Grade

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 alpha-bungarotoxin, as well as fluorescent reagents and kits for genomics and cell biology research.

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