ChromaTide® Labeled Nucleotides

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Avoid freeze–thaw cycles
- Protect from light

Concentration: 1 mM in TE buffer

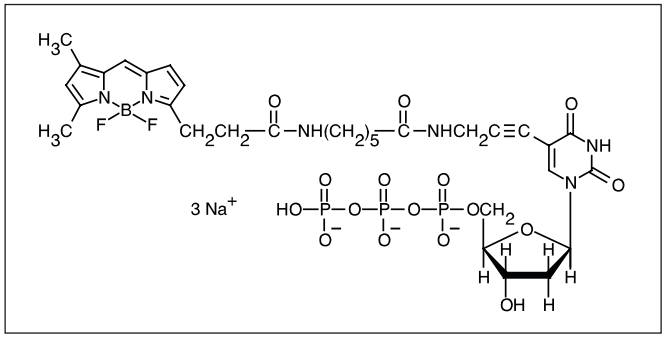

Figure 1. Structure of our ChromaTide® BODIPY® FL-14-dUTP (C7614). This structure illustrates the aminoalkynyl linker present in all of our ChromaTide® UTP and dUTP nucleotides, as well as the aminohexanoic spacer between the fluorophore and the deoxyribonucleotide, present in several of the ChromaTide® UTPs and dUTPs.

Introduction

Fluorophore-, biotin-, and hapten-labeled nucleotides offer alternatives to the hazardous and expensive radioisotope-labeled nucleotides involved in many molecular biology protocols. Molecular Probes' labeled ChromaTide® uridine, deoxyuridine, and deoxycytidine triphosphates can be used to generate labeled nucleic acid probes via standard techniques for molecular biology and molecular cytogenetics applications (see Table 1).

Probes made with labeled nucleotides can be used for multicolor techniques such as in situ hybridization and hybridization to arrays.[1-5] Biotin- or DNP-labeled nucleic acid probes can also be used in multicolor techniques by employing labeled streptavidin conjugates or labeled anti-DNP antibodies, respectively, for detection. In addition, biotin- and DNP-labeled probes have been used in a variety of molecular biology applications, including Southern and Northern blots, colony and plaque hybridizations, DNA sequencing, primer extension, DNA and RNA amplification, and bead-based separation techniques.[6] In these applications, the nonisotopically labeled samples are generally detected with streptavidin- or antibody-conjugated enzymes in conjunction with chemiluminescent or colorimetric substrates.

For multicolor applications, we recommend our proprietary BODIPY® and Alexa Fluor® dye conjugates. Both BODIPY® and Alexa Fluor® dyes are essentially pH insensitive. The narrow emission spectra of the BODIPY® dyes ensure minimal spectral overlap. The Alexa Fluor® dyes are highly water soluble, as are DNA probes made from them, making them the labels of choice for fluorescence in situ hybridization. In addition, the Alexa Fluor® dyes are exceptionally bright and photostable.

For detection of biotin or DNP, we carry an extensive line of labeled streptavidin conjugates as well as unlabeled and labeled anti-DNP antibodies. Please consult Molecular Probes' The Handbook: A Guide to Fluorescent Probes and Research Technologies or visit our website (probes.invitrogen.com) for more information.

Materials

Each ChromaTide® nucleotide is supplied as 25 µL (for dUTPs and UTPs) or 50 µL (for OBEA-dCTPs) of a 1 mM nucleotide solution in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.6). ChromaTide® nucleotides are >95% pure as determined by HPLC and spectrophotometric analysis. ChromaTide® nucleotides should be stored frozen at ≤–20°C and protected from light. When stored properly, these products will be stable for up to two years. AVOID REPEATED FREEZING AND THAWING.

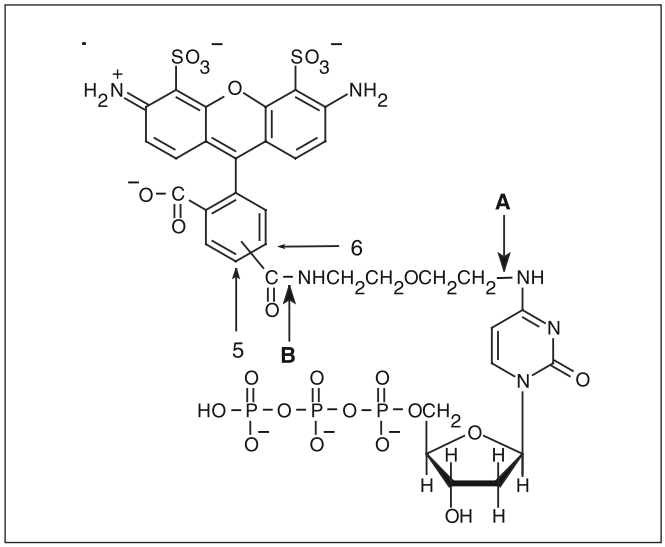

Figure 2. Structure of ChromaTide® Alexa Fluor® 488-7-OBEA-dCTP (C21555). This structure is representative of our labeled ChromaTide® OBEA-dCTP nucleotides. The fluorophores are attached via the OBEA spacer (between arrows A and B) to deoxycytidine triphosphate (dCTP).

Characteristics

Structure

The ChromaTide® dUTP and UTP nucleotides are modified at the C-5 position of uridine via a unique alkynylamino linker, which provides a spacer between the nucleotide and the dye to reduce interactions between them (Figure 1). The ChromaTide® dCTP nucleotides are modified at the N-4 position of cytosine using a 2-aminoethoxyethyl (OBEA) linker (Figure 2). Several of these nucleotides also contain an additional spacer, further separating the label from the nucleic acid. The number in the product name, e.g., the "12" in fluorescein-12-dUTP, indicates the net length of the spacer, in atoms. These spacers result in brighter conjugates and increased hapten accessibility for secondary detection reagents.

Table 1. Characteristics of ChromaTide® dUTP, dCTP, and UTP nucleotides.

Label	Ex/Em *	dUTP		OBEA-dCTP		UTP	
		Catalog #	Applications †	Catalog #	Applications †	Catalog #	Applications ‡
Blue Fluorescence							
Cascade Blue®-7-	400/420	C7612	PCR, TDT, RP				
Green Fluorescence							
BODIPY® FL-14-	505/515	C7614	PCR, TDT, RP				
Alexa Fluor® 488-5-(or 7-)	490/520	C11397	PCR, TDT, RP, RT, NT	C21555	RT, NT	C11403	SP6, T3, T7
Oregon Green® 488-5-	495/520	C7630	PCR, TDT, RP				
Fluorescein-12-	495/525	C7604	PCR, TDT, RP, NT			C7603	SP3, T3, T7
Rhodamine Green™-5-	505/530	C7629	PCR, TDT, RP			C7628	SP6, T3, T7
Yellow Fluorescence							
Alexa Fluor® 532-5-	525/550	C11398	PCR, TDT, RP, RT, NT				
Orange Fluorescence							
Tetramethylrhodamine-6-	550/570	C7606MP	PCR, TDT, RP				
Alexa Fluor® 546-14-(or 16-)	555/570	C11401	PCR, TDT, RP, RT, NT			C11404	SP6, T3, T7
Red Fluorescence							
Alexa Fluor® 568-5-	575/600	C11399	PCR, TDT, RP, NT				
Alexa Fluor® 594-5-(or 7-)	590/615	C11400	PCR, TDT, RP, NT ¶				
Texas Red® -12-	595/615	C7631	PCR, TDT, RP				
Far-Red Fluorescence							
Alexa Fluor® 647-(12-)	650/670			C21559	RT, NT		

* Excitation (Ex) and Emission (Em) maxima, in nm, for the labeled nucleotide. ** The numbers in the name indicate the number of atoms in the spacer between the nucleotide and the fluorophore or hapten for the dUTP, dCTP, or UTP nucleotides; numbers in parentheses are for the OBEA-dCTP nucleotides and include atoms in the OBEA group. † ChromaTide® dUTPs and dCTPs have been successfully incorporated into probes using the indicated methods: PCR, DNA amplification using Taq polymerase; TDT, 3′-end labeling using terminal deoxynucleotidyl transferase; RP, random hexamer–primed DNA labeling using Klenow polymerase; RT, DNA synthesized from an RNA template using murine leukemia virus (MLV) reverse transcriptase; NT, nick translation using DNase I and DNA polymerase I; § not recommended for PCR; ¶ not recommended for RT. ‡ ChromaTide® UTPs have been successfully incorporated into probes using SP6, T3, and T7 RNA polymerases, as indicated. NA, not applicable.

Spectral Properties and Filter Selection

To optimize the performance of the ChromaTide® nucleotides in your application, use a high-quality optical filter set that closely matches the spectral characteristics of the conjugates. Please note that the ChromaTide® nucleotides may undergo small spectral shifts upon incorporation into polynucleotides. Table 1 lists the approximate excitation and emission maxima determined for each of the fluorescently labeled ChromaTide® nucleotides in 50 mM phosphate buffer, pH 7.0. The full spectra for the ChromaTide® dUTP nucleotides are available at our website (probes.invitrogen.com).

References

1. Proc Natl Acad Sci USA 64, 600 (1969); 2. Nature 223, 582 (1969); 3. Nature 265, 472 (1977); 4. Exp Cell Res 138, 485 (1980); 5. Proc Natl Acad Sci USA 83, 2934 (1986); 6. Nonisotopic DNA Probe Techniques, Larry J. Kricka, Ed., Academic Press, Inc., San Diego, CA (1992).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat #	Product Name	Unit Size
C11397	ChromaTide® Alexa Fluor® 488-5-dUTP *1 mM in TE buffer* .	25 µL
C11398	ChromaTide® Alexa Fluor® 532-5-dUTP *1 mM in TE buffer* .	25 µL
C11399	ChromaTide® Alexa Fluor® 568-5-dUTP *1 mM in TE buffer* .	25 µL
C11400	ChromaTide® Alexa Fluor® 594-5-dUTP *1 mM in TE buffer* .	25 µL
C11401	ChromaTide® Alexa Fluor® 546-14-dUTP *1 mM in TE buffer*.	25 µL
C11403	ChromaTide® Alexa Fluor® 488-5-UTP *1 mM in TE buffer* .	25 µL
C11404	ChromaTide® Alexa Fluor® 546-14-UTP *1 mM in TE buffer*.	25 µL
C21555	ChromaTide® Alexa Fluor® 488-7-OBEA-dCTP *1 mM in TE buffer*.	50 µL
C21556	ChromaTide® Alexa Fluor® 546-16-OBEA-dCTP *1 mM in TE buffer* .	50 µL
C21559	ChromaTide® Alexa Fluor® 647-12-OBEA-dCTP *1 mM in TE buffer* .	50 µL
C7603	ChromaTide® fluorescein-12-UTP *1 mM in TE buffer*.	25 µL
C7604	ChromaTide® fluorescein-12-dUTP *1 mM in TE buffer*.	25 µL
C7606MP	ChromaTide® tetramethylrhodamine-6-dUTP *1 mM in TE buffer*.	25 µL
C7612	ChromaTide® Cascade Blue®-7-dUTP *1 mM in TE buffer*.	25 µL
C7614	ChromaTide® BODIPY® FL-14-dUTP *1 mM in TE buffer*.	25 µL
C7628	ChromaTide® Rhodamine Green™-5-UTP *1 mM in TE buffer*.	25 µL
C7629	ChromaTide® Rhodamine Green™-5-dUTP *1 mM in TE buffer*.	25 µL
C7630	ChromaTide® Oregon Green® 488-5-dUTP *1 mM in TE buffer* .	25 µL
C7631	ChromaTide® Texas Red®-12-dUTP *1 mM in TE buffer* .	25 µL

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Please visit our website — probes.invitrogen.com — for the most up-to-date information.

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