Labeled aha-dUTP and aha-dCTP

Quick Facts

Storage upon receipt:

- ≤–20°C

Concentration: 1 mM nucleotide solution in TE buffer (pH 8.0)

Introduction

Labeled aha-dUTP and aha-dCTP (5-aminohexylacrylamido-dUTP and -dCTP) nucleotides are modified with a unique hexylacrylamide linker at the C-5 position of uridine and cytosine, respectively. This modification serves as a spacer between the nucleotide and the dye and reduces interactions between these two portions of the molecule, resulting in brighter conjugates and increased hapten accessibility for secondary detection reagents. Labeled nucleotides can be used to generate labeled nucleic acid hybridization probes for many molecular biology and molecular cytogenetics applications, including multicolor techniques.[1–5]

The Alexa Fluor® 555 and Alexa Fluor® 647 dyes used to label these nucleotides are compatible with commonly used microarray scanners, and provide greater signal correlation (R^2) values than the spectrally similar Cy™3 and Cy™5 dye pair, improving the resolution of two-color microarray gene expression assays to 1.3-fold changes in expression.[6] The exceptionally bright and photostable Alexa Fluor® dyes are also essentially insensitive to pH and are highly water soluble.[7]

Biotin- or fluorescein-labeled nucleic acid probes are easily detected with streptavidin conjugates or labeled anti-fluorescein or anti-biotin antibodies, respectively, and have been used in two-color microarray assays, Southern and Northern blots, colony and plaque hybridizations, DNA sequencing, primer extension, DNA and RNA amplification, and bead-based separation techniques.[8] In these applications, the labeled samples are generally detected with streptavidin- or antibody-conjugated enzymes in conjunction with chemiluminescent, fluorescent, or colorimetric substrates, such as those employed in our Tyramide Signal Amplification (TSA™) Kits. Please consult our *Handbook of Fluorescent Probes and Research Products* or visit our Web site (www.probes.com) for more information.

Table 1. Labeled 5-aminohexylacrylamido-dUTP and -dCTP nucleotides.

Label	Ex/Em*	Color	Catalog Number
Biotin	NA	Nonfluorescent	B32766, B32772
Fluorescein	495/525	Green fluorescent	F32767, F32773
Alexa Fluor® 555	555/570	Orange fluorescent	A32762, A32770
Alexa Fluor® 647	650/670	Far-red fluorescent	A32763, A32771

* Excitation (Ex) and fluorescence emission (Em) maxima, in nm.

Materials

Each labeled 5-aminohexylacrylamido-dUTP nucleotide is supplied as a 1 mM nucleotide solution in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). These nucleotides are >95% pure as determined by HPLC and spectrophotometric analysis. 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 FREEZE/THAW CYCLES.

Spectral Properties and Filter Selection

For optimal performance of the nucleotides in your application, use a high-quality optical filter set that closely matches the spectral characteristics of the conjugates. Please note that these 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 nucleotides in 50 mM phosphate buffer (pH 7.0). Full spectra for the labeled 5-aminohexylacrylamido-dUTP and -dCTP nucleotides are available at our Web site (www.probes.com).

Enzymatic Incorporation Protocol

We have optimized the following reverse transcription protocol using the SuperScript™ Direct cDNA Labeling Kit and Superscript™ III reverse transcriptase (Invitrogen Corp.). Modification of this protocol may be required for use with other

systems; the most useful parameter to modify is the ratio of labeled aha-dNTP to dNTP (i.e., the ratio of aha-dUTP to dTTP or aha-dCTP to dCTP, depending on the aha-dNTP used in the reaction). **For cDNA synthesis using labeled aha-dUTP,** optimal results are obtained using 500 µM final concentration of dGTP, dATP, and dCTP, and a 3:1 mixture of labeled aha-dUTP to unlabeled dTTP to give a final combined nucleotide concentration of 300 µM (i.e. 200 µM labeled aha-dUTP and 100 µM dTTP). **For cDNA synthesis using labeled aha-dCTP,** optimal results are obtained using 500 µM final concentration of dGTP, dATP, and dTTP, and a 3:1 mixture of labeled aha-dCTP to unlabeled dCTP to give a final combined nucleotide concentration of 300 µM (i.e. 200 µM labeled aha-dCTP and 100 µM dCTP).

Materials Required

- Labeled aha-dNTP, 1 mM solution (provided)
- 10–40 µg of total RNA
- SuperScript™ III Reverse transcriptase (400 U/µL)
- d(GACT) mixture containing:
  7.5 mM each of dGTP, dATP, dCTP, and 1.5 mM of dTTP (**for reactions incorporating labeled aha-dUTP**)

OR

  7.5 mM each of dGTP, dATP, dTTP, and 1.5 mM of dCTP (**for reactions incorporating labeled aha-dCTP**)
- Anchored Oligo (dT)20 Primer, 2.5 mg/mL
- 5X first-strand buffer for reverse transcriptase (250 mM Tris, 375 mM KCl, 15 mM $MgCl_2$, pH 8.3)
- 100 mM DTT
- RNaseOUT™ (40 U/µL)

Labeling Reaction

1. Add dH_2O to the sample of RNA to bring the final volume to 8 µL.

2. Add 2 µL of primer and mix thoroughly.

3. Heat the template and primer mixture to 70°C for 10 minutes and then transfer immediately to ice.

4. To the tube containing the denatured template and primers, add:
- 6 µL 5X first-strand buffer
- 3 µL 100 mM DTT
- 2 µL d(GACT)
- 1 µL RNaseOUT™
- 6 µL Labeled aha-dNTP (1 mM solution)
- 2 µL SuperScript™ III

5. Mix gently and incubate the reaction at 25°C for 10 minutes, followed by a three-hour incubation at 46°C.

6. After cDNA synthesis, above, immediately perform the following hydrolysis reaction to degrade the RNA. Place the reverse transcription reaction at 95°C for 5 minutes to inactivate the reverse transcriptase and denature the RNA:cDNA hybrids. Snap cool by immediately placing the reaction vial into an ice bath. Add 15 µL of 0.1M NaOH to the reaction, mix thoroughly, and incubate at 65°C for 15 minutes. Add 15 µL of 0.1M HCl to neutralize the pH and mix gently.

Purification of RT-Labeled DNA

Purify the labeled DNA using a Qiagen QIAQuick™ PCR Purification Kit using the manufacturer's protocol, followed by ethanol precipitation.

References

1. Proc Natl Acad Sci U S A 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 U S A 83, 2934 (1986); **6.** Anal Biochem 331, 243 (2004); **7.** J Histochem Cytochem 47, 1179 (1999); **8.** *Nonisotopic DNA Probe Techniques*, Larry J. Kricka, Ed., Academic Press, Inc., San Diego, CA (1992).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
A32770	Alexa Fluor® 555-aha-dCTP *1 mM in TE buffer*	50 µl
A32762	Alexa Fluor® 555-aha-dUTP *1 mM in TE buffer*	50 µl
A32771	Alexa Fluor® 647-aha-dCTP *1 mM in TE buffer*	50 µl
A32763	Alexa Fluor® 647-aha-dUTP *1 mM in TE buffer*	50 µl
B32772	biotin-aha-dCTP *1 mM in TE buffer*	25 µl
B32766	biotin-aha-dUTP *1 mM in TE buffer*	25 µl
F32773	fluorescein-aha-dCTP *1 mM in TE buffer*	50 µl
F32767	fluorescein-aha-dUTP *1 mM in TE buffer*	50 µl

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