cytiva

Amersham Cy5-dUTP

5-Amino-propargyl-2′-deoxyuridine 5′-triphosphate coupled to Cy5 fluorescent dye (Cy5-AP3-dUTP)

Product Booklet

Table of Contents

1 Introduction

Product code

PA55022

Components

25 nmoles Cy™5-dUTP in 10 mM phosphate buffer, pH 7.0.

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage

Store at -15°C to -30°C in the dark. Do not store below -30°C. Light-sensitive material. See *Chapter 3 Important information and tips on handling CyDye products, on page 6*.

Expiry

See outer packaging.

2 Background

The CyDye™ fluors from Cytiva have gained world-wide acceptance as exceptional dyes for fluorescence analysis such as microarray detection. Highly fluorescent and water soluble they provide significant advantages over existing fluorescent labels and are ideal for the non-radioactive labeling of DNA probes, oligonucleotides and other nucleic acids.

- **Bright, intense dyes** - CyDye fluors give bright signals increasing the amount of information generated.
- **High sensitivity** - meaningful data can be generated from low copy number transcripts.
- **Spectrally resolved dyes** - for multiplexing, give discreet, discernible signals. The CyDye absorption and emission spectra are sharp, resulting in good spectral separation of the different fluors.

They exhibit:

- Low non-specific binding
- High photo stability
- High water solubility
- pH insensitivity
- Survive harsh hybridization conditions

Cy5 labeled dUTP has spectral characteristics similar to Tetramethylrhodamine and can be used with many existing rhodamine filter sets.

Cy5-dUTP characteristics

Absorbance max	649 nm
Extinction max	250 000 $M^{-1}cm^{-1}$
Emission max	670 nm
Quantum yield	>0.28

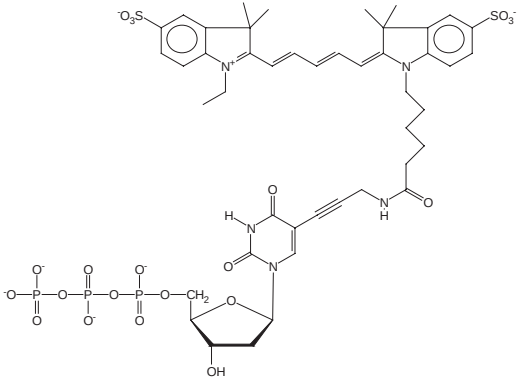

Fig 1. Cy5-AP3-dUTP

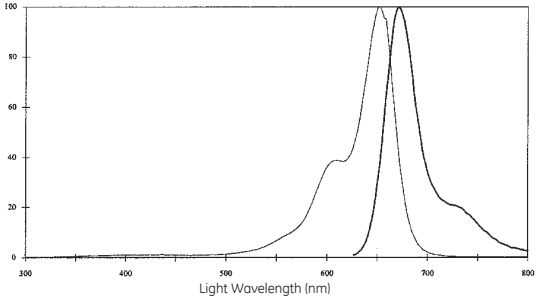

Fig 2. Cy5 fluorescent dye absorption and fluorescence spectra

3 Important information and tips on handling CyDye products

CyDye products are excellent fluors in many applications. However, their fluorescence is sensitive to environmental conditions and we advise that precautions are taken when handling.

Please follow these precautions when handling Cy-labeled nucleotides and when setting up labeling reactions:

1. **Protect Cy-labeled dNTPs from light at all times. All exposures to light must be minimal and restricted to the time taken to complete a required operation. As far as possible, carry out experimental procedures in low light conditions.**

Photostability of Cyanine dyes decreases as the distance between the two indolenine moieties of the chromophore increases. Thus Cy7 is much less stable than Cy2 and Cy5 is less stable than Cy3. In tests at Cytiva laboratories Cy5 dye arrayed onto a microarray slide loses about 50% of its fluorescence after 24 hours exposure to laboratory strip lighting [sunlight excluded]. Repeated red laser scans cause a small but detectable signal loss amounting to <1% per scan. Photostability differences between Cy3 and Cy5 will not normally present a problem if the guidelines above are followed. Remember also to perform hybridizations and stringency washes in the dark as high temperatures accelerate photodestruction of the fluorophore.

2. **Store Cy-labeled nucleotides at -15°C to -30°C in the dark. Dispense all Cy-dNTPs into single-use aliquots to minimize freeze-thaw cycles.**

   Nucleotide solutions are stable for many years if kept frozen at -15°C to -30°C. When subjected to repeated rounds of freezing and thawing the phosphate bonds are progressively broken. The product is chiefly nucleoside mono-phosphate. Labeling efficiency gradually declines since mono-phosphates are not polymerase substrates.

3. **Take care in handling; wear gloves at all times.**

   Keep inside a lightproof container to protect from refrigerator lights. dNTPs and rNTPs are stable for up to 3 months at 2–8°C in the absence of divalent cations and at a slightly alkaline pH of ~8.

4. **Treat labeled probes with the same care.**

Nucleases and phosphatases from unguarded fingers degrade nucleotides, mRNA and DNA probes. Powder and talc from some brands of glove will bind nucleic acids and are also highly fluorescent.

5. **Minimize exposure to amine containing solutions.**

   The CyDye products are sensitive to nitrogen bases including primary and secondary amines, especially organic amines, in basic conditions (pH 8 and above). You should therefore minimize their exposure to Tris buffers and other amine containing solutions.

Change to the manufacturing process of Cy-nucleotides

We have reviewed the manufacturing process for Cy-nucleotides to ensure that exposure to light is avoided or minimized at all stages of manufacture. All nucleotides are supplied in black securitainers. We recommend that you store Cy-nucleotides in the securitainer provided to reduce exposure to light (eg from lights in freezers) and also minimize the exposure to repeated freeze-thawing by dispensing your stock into experimental quantities.

In common with other fluors, CyDye products undergo oxidative degradation with time. For this reason we recommend the rapid handling of experimental material and observation of the above precautions.

Microarray recommendations

Cy3 and Cy5 have become the labels of choice for microarray detection because they offer bright and intense colors with narrow emission bands. These two dyes are spectrally distinct, making them ideal for dual color detection in microarrays. There are numerous methods for preparing a fluorescently

labeled probe such as cDNA labeling, random prime labeling and nick translation. For microarray detection the range of CyScribe microarray labeling kits offers the choice between first strand cDNA synthesis, post-labeling of cDNA and direct mRNA labeling (see *Chapter 5 Related products, on page 11*).

4 Protocol - Nick translation

Other materials required

- DNA to be labeled, digested to the desired size and nicked with DNase I
- Unlabeled dATP, dCTP, dGTP (200 µM solutions adjusted to pH 7.0)
- *E.coli* DNA polymerase I (10 U/µl)
- 10 × nick translation buffer

  0.5 M Tris-HCl (pH 7.2)

  0.1 M $MgSO_4$

  1 mM Dithiothreitol
- 10 M Ammonium Acetate
- Cold 100% Ethanol (2–8°C)
- Cold 70% Ethanol (2–8°C)
- TE buffer (pH 8.0)

  10 mM Tris-HCl

  0.1 mM EDTA
- Deionized water

Nick translation procedure

Step	Action
1	Combine the following in a 1.5 ml microcentrifuge tube in an ice water bath: deionized H_2O to a final volume of 250 µl 25 µl 10 × nick translation buffer 25 µl each unlabeled dATP, dCTP, dGTP 5 µl Cy3-dUTP 10 µg DNase I digested DNA 10 µl DNA polymerase I, 10 U/µl
2	Mix thoroughly.
3	Incubate mixture at 15°C for at least 2 hours. Mixture may be incubated overnight if required.
4	Precipitate the labeled DNA by adding: 83 µl 10 M Ammonium Acetate 667 µl cold 100% Ethanol
5	Precipitate in an ice water bath for at least one hour, or overnight if required.
6	Spin at 12 000–16 000 × g for 30 minutes at 4°C.
7	Rinse twice with cold 70% Ethanol.
8	Spin under vacuum until almost dry.

Step	Action
9	Resuspend in 100 µl TE buffer (pH 8.0), mixing thoroughly. Incubate at 37°C for 20 minutes if necessary to dissolve completely.
10	Store at -15°C to -30°C until ready for use.

Emission spectra of CyDye products

Fluorophore	Color of fluorescence	Absorption maximum (nm)	Fluorescence maximum (nm)	Extinction coeff ($M^{-1}cm^{-1}$)	Formula weight (Daltons) Bisfunc.	Formula weight (Daltons) Monofunc.
Cy2	Green	489	506	~150000	896.95	713.78
Cy3	Orange	550	570	150000	949.11	765.95
Cy3.5	Scarlet	581	596	150000	1285.54	1102.37
Cy5	Far-Red	649	670	250000	975.15	791.99
Cy5.5	Near IR	675	694	250000	1311.58	1128.41
Cy7	Near IR	743	767	~250000	1001.19	818.02
FluorX	Green	494	520	68000		586.60

5 Related products

CyDye fluors are available as CyDye-deoxynucleotides, CyDye ribonucleotides, highly reactive NHS esters, Cy Direct Labeling reagents and CyScribe microarray labeling kits.

CyDye nucleotides		
Cy3-dCTP	25 nmol	PA53021
Cy5-dCTP	25 nmol	PA55021
Cy3-dUTP	25 nmol	PA53022
Cy5-dUTP	25 nmol	PA55022
Cy3.5-dCTP	25 nmol	PA53521

Cy5.5-dCTP	25 nmol	PA55521
Cy3-UTP	100 nmol	PA53026
Cy5-UTP	100 nmol	PA55026
CyDye value packs		
Cy3 dCTP 25 nmol × 5 plus Cy5 dCTP 25 nmol × 5		PA55321
Cy3 dUTP 25 nmol × 5 plus Cy5 dUTP 25 nmol × 5		PA55322
CyScribe First-Strand cDNA Labeling Kit	25 reactions	RPN6200
CyScribe First-Strand cDNA Labeling Kit with CyScribe GFX Purification Kit	25 reactions	RPN6200X
CyScribe First-Strand cDNA Labeling System with 25 nmol Cy3 dUTP and 25 nmol Cy5 dUTP	50 reactions	RPN6201
CyScribe First-Strand cDNA Labeling System with 25 nmol Cy3 dUTP and 25 nmol Cy5 dUTP with CyScribe GFX Purification Kit	50 reactions	RPN6201X
CyScribe First-Strand cDNA Labeling System with 25 nmol Cy3 dCTP and 25 nmol Cy5 dCTP	50 reactions	RPN6202
CyScribe First-Strand cDNA Labeling System with 25 nmol Cy3 dCTP and 25 nmol Cy5 dCTP with CyScribe GFX Purification Kit	50 reactions	RPN6202X
CyScribe Post-Labeling Kit 12 of Cy3 and 12 of Cy5	24 reactions	RPN5660
CyScribe Post-Labeling Kit 12 of Cy3 and 12 of Cy5 with Cyscribe GFX Purification Kit	24 reactions	RPN5660X
CyDye Post Labeling Reactive Dye Pack 12 of Cy3 and 12 of Cy5	24 reactions	RPN5661

CyScribe Direct mRNA Labeling Kit 12 of Cy3 and 12 of Cy5	24 reactions	RPN5665
Microarray experiment validation Lucidea Microarray ScoreCard Kit v1.1 Human Includes analysis software for normalizing and validating data, control plate and spike mix for labeling and hybridization		RPK1161
Lucidea Microarray ScoreCard Kit v1.1 Mouse Probe Quantification		RPK1165
Ultrospec™ 3100Pro		80-2112-31/32/37/38

6 References

1. Mujumdar, R.B. et al., Bioconjugate Chemistry 4 (2), 105-111 (1993).
2. Yu, H. et al., Nucl. Acids Res. 22, 3226-3232 (1994).
3. Zhu, Z. et al., Nucl. Acids Res. 22, 3418-3422 (1994).

cytiva.com

For local office contact information, visit cytiva.com/contact

PA55022PL AE V:4 03/2021