



# Amersham Cy3-dUTP

Product booklet

# Table of Contents

1	Introduction .....	3
2	Background .....	4
3	Cy3-dUTP characteristics .....	5
4	Important information and tips on handling CyDye products .....	6
5	Protocol - Nick translation .....	9
6	Related products .....	11
7	References .....	14

# 1 Introduction

## Product code

PA53022

## About

5-Amino-propargyl-2'-deoxyuridine 5'-triphosphate coupled to Cy<sup>™</sup>3 fluorescent dye (Cy3-AP3-dUTP).

## 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 4 Important information and tips on handling CyDye products, on page 6](#).

## Expiry

See outer packaging.

## Components

25 nmoles Cy3-dUTP in 10 mM phosphate buffer, pH 7.0.

## 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

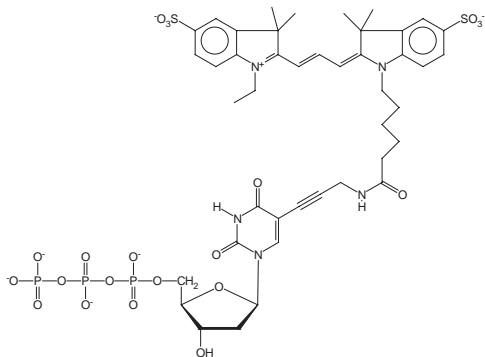
They exhibit:

- low non-specific binding
- high photo stability
- high water solubility
- pH insensitivity
- survive harsh hybridization conditions

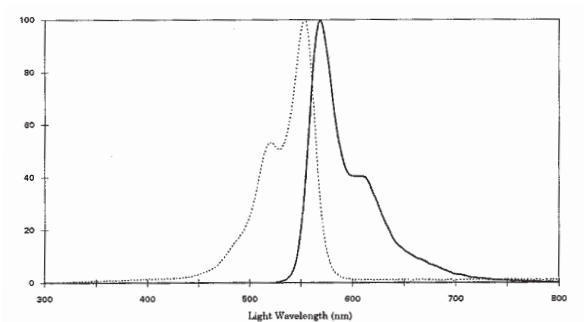
*Cy3 labeled dUTP has spectral characteristics similar to Tetramethylrhodamine and can be used with many existing rhodamine filter sets.*

### 3 Cy3-dUTP characteristics

Absorbance max	550 nm
Extinction max	150 000 M <sup>-1</sup> cm <sup>-1</sup>
Emission max	570 nm
Quantum yield	>0.15



**Fig 1.** Cy3-AP3-dUTP



**Fig 2.** Cy3 fluorescent dye absorption and fluorescence spectra

## 4 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:

- 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.

- 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.
- 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.
- 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.

- 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 6 Related products, on page 11](#)).

## 5 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  $\mu$ M solutions adjusted to pH 7.0)
- *E.coli* DNA polymerase I (10 U/ $\mu$ L)
- 10  $\times$  nick translation buffer
  - 0.5 M Tris-HCl (pH 7.2)
  - 0.1 M MgSO<sub>4</sub>
  - 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
------	--------

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

## Step Action

- Resuspend in 100  $\mu$ L TE buffer (pH 8.0), mixing thoroughly. Incubate at 37°C for 20 minutes if necessary to dissolve completely.
- Store at -15°C to -30°C until ready for use.

## Emission spectra of CyDye products

Fluorophore	Colour of fluorescence	Absorption maximum	Fluorescence maximum	Extinction coeff. ( $M^{-1}cm^{-1}$ )	Formula weight	Formula weight
		(nm)	(nm)		(Daltons) Bisfunc.	(Daltons) Monofunc.
Cy2	Green	489	506	~150 000	896.95	713.78
Cy3	Orange	550	570	150 000	949.11	765.95
Cy3.5	Scarlet	581	596	150 000	1285.54	1102.37
Cy5	Far-Red	649	670	250 000	975.15	791.99
Cy5.5	Near IR	675	694	250 000	1311.58	1128.41
Cy7	Near IR	743	767	~250 000	1001.19	818.02
FluorX	Green	494	520	68 000		586.60

## 6 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		Product code
Cy3-dCTP	25 nmol	PA53021
Cy5-dCTP	25nmol	PA55021

<b>CyDye nucleotides</b>		<b>Product code</b>
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	PA53026

<b>Microarray experiment validation</b>	<b>Product code</b>
Lucidea Microarray ScoreCard Kit v1.1 Human	RPK1161
Includes analysis software for normalizing and validating data, control plate and spike mix for labeling and hybridization.	
Lucidea Microarray ScoreCard Kit v1.1 Mouse	RPK1165

<b>Probe Quantification</b>	<b>Product code</b>
Ultrospec™ 3100Pro	80-2112-31/32/37/38

CyDye value packs		Product code
Cy3 dCTP 25 nmol x 5 plus Cy5 dCTP	25 nmol x 5	PA55321
Cy3 dUTP 25 nmol x 5 plus Cy5 dUTP	25 nmol x 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

## 7 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).

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