

GE Healthcare

Amersham Cy3-dCTP

5-Amino-propargyl-2'-deoxycytidine 5'-triphosphate coupled
to Cy3 fluorescent dye (Cy3-AP3-dCTP)

Product Booklet

Code: 28-9231-85



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1. Legal

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Cy3-dUTP or Cy5-dUTP, Cy3.5-dCTP or Cy5.5-dCTP, Cy3-dCTP or Cy5-dCTP: These products are manufactured for GE Healthcare UK by

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GE Healthcare UK Limited.

Amersham Place, Little Chalfont,

Buckinghamshire, HP7 9NA UK

2. Handling

2.1. Safety warnings and precautions

Warning: For research use

only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Store at -15°C to -30°C in the dark. Do not store below -30°C. Light-sensitive material. See important information and tips on handling CyDye products.

2.3. Expiry

See outer packaging.

3. Components

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

4. Introduction

The CyDye™ fluors from GE Healthcare 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 labelling 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

Cy™3 labelled dCTP has spectral characteristics similar to Tetramethylrhodamine and can be used with many existing rhodamine filter sets.

To complement CyDye fluors, a new class of microarray dyes known as HyPer5™ have been developed that demonstrate high photo - and ozone-stability and give consistent array performance irrespective of environmental conditions. Like Cy3 dye, HyPer5 dyes are resilient to repeat array scanning and do not degrade when

exposed to high ozone levels that sometimes prevail in summer months. The HyPer5-dCTP can be used in conjunction with Cy3-dCTP in enzymatic incorporation reactions to make probes for array detection and analysis.

Cy3-dCTP characteristics

Absorbance max	550 nm
Extinction max	150 000 M ⁻¹ cm ⁻¹
Emission max	570 nm
Quantum yield	> 0.15

Figure 1. Cy3-AP3-dCTP

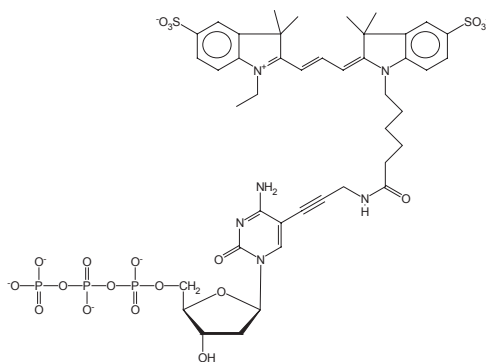
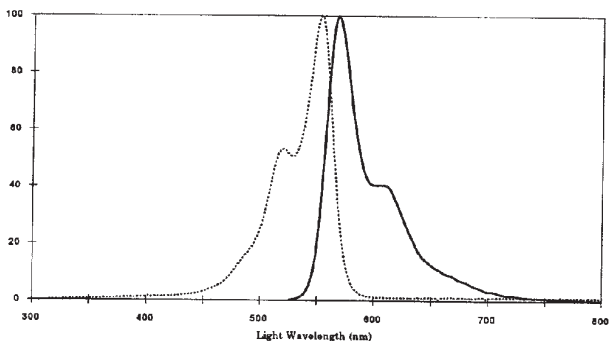


Figure 2. Cy3 fluorescent dye absorption and fluorescence spectra



Dashed line = absorbance

Solid line = fluorescence

5. 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 during handling.

Please follow these precautions when handling Cy-labelled nucleotides and when performing labeling reactions:

1. Protect Cy-labelled 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 GE Healthcare 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. Remember also to perform hybridizations and stringency washes in the dark as high temperatures accelerate photodestruction of the fluorophore.

2. Store Cy-labelled 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. Labelling 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 labelled 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 HyPer5 dyes can be used in conjunction for microarray probe synthesis and array detection. HyPer5 dye is spectrally distinct from Cy3, making them suitable for dual color detection in microarrays. Typically 1-5 μ l of Cy3-or HyPer5-dCTP is sufficient to generate probes for array applications. Depending on the concentration of unlabeled dCTP in the labeling mixture, the amount of Cy3 or HyPer5 may need optimization for high dye incorporation.

6. Protocol- Nick translation

1. Other materials required

- DNA to be labelled, digested to the desired size and nicked with DNase I
- Unlabelled dATP, dGTP, dTTP (200 μ M solutions adjusted to pH 7.0)
- *E.coli* DNA polymerase I (10 U/ μ l)
- 10 $\times$ 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

2. Nick translation procedure

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 μ l
25 μ l 10 $\times$ nick translation buffer
25 μ l each unlabelled dATP, dGTP, dTTP
5 μ l Cy3-dCTP
10 μ g DNase I digested DNA
10 μ l DNA polymerase I, 10 U/ μ l
2. Mix thoroughly.
3. Incubate mixture at 15°C for at least 2 hours. Mixture may be incubated overnight if required.

4. Precipitate the labelled 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.
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.

7. Additional information

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	~ 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

7.1. 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).

7.2. Related products

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

HyPer5 dCTP	25 nmol	28923183
HyPer5 dCTP	250 nmol	28923184

Cy3-dCTP	25 nmol	PA53021
Cy3.5-dCTP	25 nmol	PA53521
Cy5-dCTP	25 nmol	PA55021

Cy5.5-dCTP	25 nmol	PA55521
Cy3-dUTP	25 nmol	PA53022
Cy5-dUTP	25 nmol	PA55022
Cy3-dCTP	250 nmol	PA53031
Cy5-dCTP	250 nmol	PA55031
Cy3-dUTP	250 nmol	PA53032
Cy5-dUTP	250 nmol	PA55032
HyPer5 Post-Labeling Reactive Dye Pack		28-9224-18
Cy3 and HyPer5 Post-Labeling Reactive Dye Pack		28-9224-19

CyDye value packs

Cy3-dCTP	25 nmol × 5 plus HyPer5 dCTP 25 nmol × 5	28923185
Cy3-dCTP	25 nmol × 5 plus Cy5 dCTP 25 nmol × 5	PA55321
Cy3-dUTP	25 nmol × 5 plus Cy5-dUTP 25 nmol × 5	PA55322
Amersham CyScribe Array CGH Genomic DNA Labeling System		28919956
CyScribe First-Strand cDNA Labelling Kit	25 reactions	RPN6200
CyScribe First-Strand cDNA Labelling Kit with CyScribe GFX Purification Kit	25 reactions	RPN6200X
CyScribe First-Strand cDNA Labelling System with 25 nmol Cy3 dUTP and 25 nmol Cy5 dUTP	50 reactions	RPN6201
CyScribe First-Strand cDNA Labelling System with 25 nmol Cy3 dUTP and 25 nmol Cy5 dUTP with CyScribe GFX Purification Kit	50 reactions	RPN6201X
CyScribe First-Strand cDNA Labelling System with 25 nmol Cy3 dCTP and 25 nmol Cy5 dCTP	50 reactions	RPN6202

CyScribe First-Strand cDNA Labelling 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 Labelling Reactive Dye Pack 12 of Cy3 and 12 of Cy5	24 reactions	RPN5661
CyScribe Direct™ mRNA Labelling Kit 12 of Cy3 and 12 of Cy5	24 reactions	RPN5665

Microarray experiment validation

Lucidea™ Microarray ScoreCard™ Kit v1.1 Human RPK1161
Includes analysis software for normalizing and validating data, control plate and spike mix for labelling and hybridization

Lucidea Microarray ScoreCard Kit v1.1 Mouse RPK1165

Probe Quantification

Ultrospec™ 3100Pro 80-2112-31/32/37/38

Microarray Hybridization Buffer RPK0325

GE Healthcare offices:

GE Healthcare Bio-Sciences AB
Björkgatan 30, 751 84 Uppsala,
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg,
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327,
Piscataway, NJ 08855-1327,
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1, Hyakunincho,
Shinjuku-ku, Tokyo 169-0073,
Japan

<http://www.gehealthcare.com/lifesciences>

GE Healthcare UK Limited
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA
UK



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