Amersham **Cy**5-UTP

Cy5 aminoallyl-UTP

Product Specification Sheet

Introduction

Product code

PA55026

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.

Materials supplied

100 nmoles Cy™5-17-UTP, in 10 mM phosphate buffer, pH 7.0 5 mM concentration.

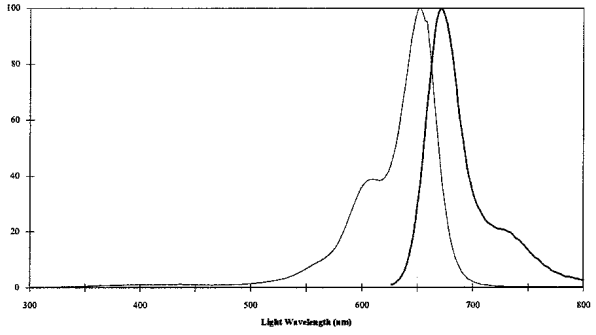

Fig 1. Cy5 fluorescent dye absorption and fluorescence spectra

Background

Cyanine dyes are useful as fluorescent labels for biological compounds (1). These dyes are both highly fluorescent and water soluble, providing significant advantages over existing fluorescent labels and are ideal for non-radioactive labeling of RNA probes.

The Cy5-labeled UTP fluoresces in the far-red region of the spectrum. Compounds labeled with the Cy5 dye are ideal for experiments where background from cellular components (such as pigments) is a problem. Cy5 dye is an ideal second dye for multicolour analyses using imaging equipment.

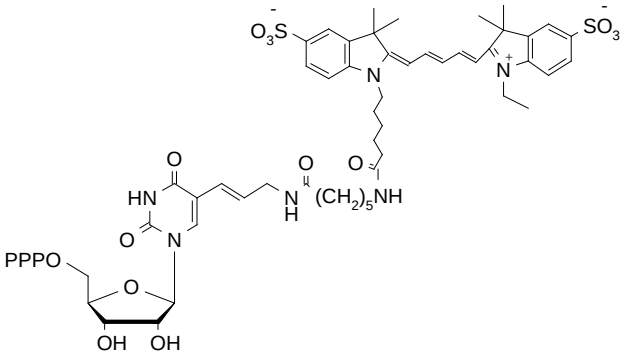

Fig 2. Cy5-17-UTP

Important information and tips on handling CyDye products

The CyDye™ products are excellent fluors in solution assays and gel electrophoresis. However, their fluorescence is sensitive to environmental conditions and we advise that precautions are taken when handling.

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

Protect Cy-labeled NTPs from light at all times

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.

Note: *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.*

Store Cy-labeled nucleotides at -15°C to -30°C in the dark

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.

Note: *Dispense all Cy-NTPs into single-use portions to minimize freeze-thaw cycles.*

Take care in handling; wear gloves at all times

Keep inside a lightproof container to protect from refrigerator lights. rNTPs are stable for up to 3 months at 2°C–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.

Changes 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 this securitainer to cut down on exposure to light (e.g. 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.

Cy5-UTP characteristics

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

Additional reagents required

- RNA labeling kit; for example, Ambion, T7 MEGAscript™ in vitro transcription kit: Product code 1334
- T7 RNA polymerase (100U/l); for example, Cytiva: Product code E70001Y
- RNA purification system; for example, QIAGEN™, RNeasy™ mini kit purification columns: Product code 714103

Protocol for the synthesis of Cy5-UTP labeled RNA

This protocol contains the information required to synthesize, purify and quantify Cy5-labeled RNA from an in vitro transcription reaction using a double stranded DNA template containing a suitable T7 RNA polymerase promoter site.

Take appropriate precautions to protect all reagents from nucleases and minimize the exposure to light during use and storage of both Cy5-UTP and Cy5-labeled RNA. Use RNase-free or DEPC treated water throughout these protocols.

Labeling reaction

Step	Action
1	Place the required tubes from an RNA labeling kit, excluding the enzyme, on ice to thaw. Protect the Cy5-UTP from exposure to light. The double stranded DNA template should be supplied at a concentration of 0.5-1 µg/mL. ***Note:*** *The Ambion MEGAscript kit is recommended. This contains 75 mM stocks of the unlabeled ribonucleotides, a 10× reaction buffer and an enzyme mix containing T7 RNA polymerase.*
2	Make a 50 mM UTP stock solution. Store on ice until use.
3	Make an ATP/CTP/GTP stock solution containing 25 mM of each ribonucleotide. Store on ice until use.
4	Add the reagents, in the following order, to a nuclease-free, 1.5 ml conical polypropylene tube: Reaction buffer 2 µL A/C/G stock solution 6 µL U stock solution 2 µL Water 4 µL Cy5-UTP solution 6 µL (for example, USB™ RNase-free H_2O: Product code US70783)
5	Mix well by pipetting up and down in the pipette tip. ***Note:*** *Ensure that all the Cy5-UTP is removed from the pipette tip and dispersed through the reaction mix.*
6	Add the DNA template, enzyme mix and T7 RNA polymerase. ***Note:*** *Mix gently to avoid denaturing the enzymes. Extra T7 RNA polymerase is also recommended to boost the yield of CyDye labeled RNA.*
7	Incubate the reactions at 37°C for 4–6 hours in a waterbath, in the dark. ***Note:*** *Check at intervals and spin briefly if there is a lot of condensation on the tube walls. Return the tube promptly to the waterbath to complete the incubation.*
8	Remove the template DNA by addition of 1 µL of RNase-free DNase. Mix gently, microcentrifuge and incubate at 37°C for 15 minutes. ***Note:*** *The Ambion MEGAscript kit contains RNase-free DNase.*

Purification of probes

Step	Action
1	Follow the QIAGEN RNeasy mini protocol for RNA clean-up. Use two QIAGEN RNeasy columns per reaction, or purify half the total volume on 1 column. Store unpurified probes at -70°C in the dark. ***Note:*** *These columns efficiently remove free Cy5-UTP and have a capacity of ~100 µg for unlabeled RNA but overloading can occur with significantly lower amounts of CyDye labeled RNA. This can result in free CyDye in the purified probes and overestimation of probe 'specific activity'.*
2	Apply the sample and collect the eluent. Reload the eluent on to the column and discard the eluent after this second loading. ***Note:*** *Once the column has been loaded make sure that the procedure is finished without any breaks. Make up just enough RLT buffer for the samples as this has a short shelf life.*
3	To elute apply 50 µL of RNase-free water. Leave for 1 minute before centrifugation. ***Note:*** *The eluent is usually highly coloured.*
4	Elute again with 30 µL of RNase-free water. Leave for 1 minute before centrifugation.
5	Combine the two eluent samples and add RNase-free 0.5 mM Na-EDTA, pH 8.0 to a final concentration of 20 mM. ***Note:*** *For example, USB Product code 15694.*
6	Keep probes on ice at all times, protected from light. Store purified probes at -70°C, in the dark.

Measurement of CyDye labeled RNA quantity

If possible use a scanning UV/visible spectrophotometer and low volume quartz cells (<100 µL volume) to avoid using too much sample.

The Ultrospec 3000, 3000 pro and 4000 UV/visible spectrophotometers with optional low volume cells are ideal for this application (contact your local office or visit the spectroscopy and fluorimetry zone through the catalogue link on the Cytiva website).

Step	Action
1	Baseline the machine against H_2O.
2	Add enough probe to achieve a 256 nm reading of at least 0.1 OD units (for example; add 2 µL probe to 98 µL of water). To increase the reading, add more probe, mix and rescan. Scan from 750–200 nm for Cy5 RNA probes.
3	Estimate the RNA concentration from the 256 nm readings: 256nm reading × dilution factor = $OD_{256\,nm}$ Assuming 1 $OD_{256\,nm}$ = 40 ng/µL RNA $OD_{256\,nm}$ × 40 = concentration of synthesized RNA (ng/µL)
	Note: *Scans are more informative than single wavelength UV measurements. The CyDye to RNA ratio (650 nm:256 nm) gives an indication of the success of the reaction and can be used to estimate the 'specific activity' of the probes if there is little free CyDye present. (Cy5 molar extinction coefficient at 650 nm = 250 000).*

Assessment of CyDye labeled RNA quality

It is recommended that labeled RNA samples are separated and imaged in denaturing agarose gels (for example glyoxal treated) to check for successful dye incorporation and purification.

The Cy5-labeled RNA and any free Cy5-UTP can be viewed directly by imaging on a suitable flat bed fluorescence scanner, for example a Typhoon™ (Cytiva) which efficiently collects the signal from bothCy3 and Cy5.

If size information of the labeled transcripts is also required, load an RNA ladder on the gel. After visualization of direct fluorescence, stain the gel for 30 minutes in a 1/10 000 dilution of Vistra Green gel stain (Cytiva): Product code RPN5786) in a fresh aliquot of the electrophoresis buffer. This stain can be imaged on the FluorImager 595 (Cytiva) and also at lower sensitivity on conventional UV transilluminators.

Storage of CyDye labeled RNA probes

Store at -70°C in the dark. Before use, thaw and keep on ice. Protect from light as much as possible during use in any subsequent application.

Emission spectra of Cy Dye products

Fluorophore	Colour 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	1 285.54	1 102.37
Cy5	Far-Red	649	670	250 000	975.15	791.99
Cy5.5	Near IR	675	694	250 000	1 311.58	1 128.41
Cy7	Near IR	743	767	~250 000	1 001.19	818.02

Related products

FluoroLink Cy5-dCTP	25 nmol	PA55021
FluoroLink Cy3.5-dCTP	25 nmol	PA53521
FluoroLink Cy5.5-dCTP	25 nmol	PA55521
FluoroLink Cy3-dUTP	25 nmol	PA53022
FluoroLink Cy5-dUTP	25 nmol	PA55022
FluoroLink Cy3-dUTP	25 nmol	PA53021

Reference

1. Mujumdar, R.B. *et al., Bioconjugate Chemistry*, **4** (2), 105-111, (1993).

cytiva.com

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

29047107 AH V:7 01/2021

cytiva