

Instruction Manual

| Product Name | Product Description | Size | Catalog Number |
|--------------|--|--|--|
| Cy5-dUTP | Red-fluorescent DNA labeling by PCR & Nick Translation | 10 μ l (1 mM) 5 x 10 μ l (1 mM) | PK-PFCY5-DUTP-10P PK-PFCY5-DUTP-50P |

Product Description

Cy5-dUTP is recommended for direct enzymatic labeling of DNA. The dye-dUTP is specially optimized for incorporation into DNA e.g. by PCR or Nick Translation using Taq polymerase or DNA Polymerase I and DNase I. It is incorporated as substitute for its natural counterpart dTTP. The excellent stability and quantum yield of the fluorophore combined with a high incorporation rate of the dye-dUTP complex makes it the ideal choice for a broad range of fluorescence applications. Optimal substrate properties and thus labeling efficiency is ensured by an optimized linker attached to the C5 position of uridine.

With our linker-optimized Cy5-modified dUTPs (dUTP-XX-Cy5) that possess a longer linker higher labeling efficiencies/rates at similar substitution/incorporation rates can be achieved compared to the previous Aminoallyl-dUTP-Cy5.

In PCR labeling, repeated cycles of denaturation, annealing and extension allow the amplification of a specific DNA fragment. The target DNA is denatured by heating followed by annealing of primers. Extension of the annealed primers with Taq polymerase results in a duplication of the DNA fragment in each cycle. When dTTP is partially substituted by dye-dUTP a fluorescent labeled double-stranded DNA is generated.

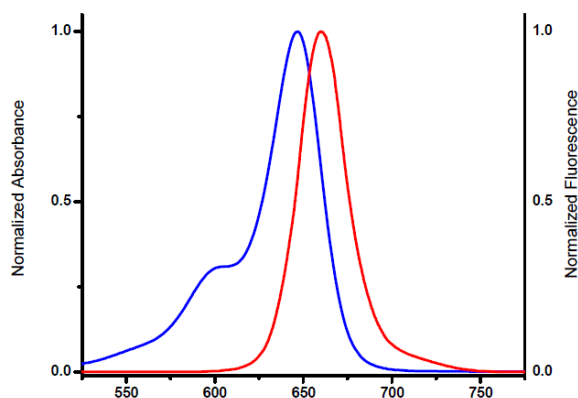
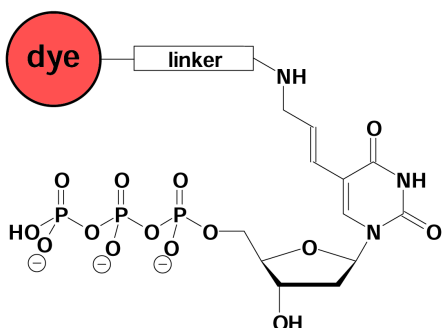
Nick translation labeling is based on the reverse activities of Polymerase I and DNase I. DNase I is able to introduce randomly distributed nicks to double stranded DNA at low enzyme concentrations. The 5'→3' exonuclease activity of Polymerase I removes nucleotides from the 3' side of the nick while synthesizing a partial new complementary strand using the 3'-OH termini as primer. In presence of dye-labeled dUTP the Polymerase I incorporates labeled dUTP instead of dTTP. Well balanced polymerase / nuclease activities in the labeling assay are required for generation of highly labeled double stranded DNA fragments.

The resulting Dye-labeled DNA/cDNA probes are ideally suited for application in FISH, microarray-based gene expression profiling and other nucleic acid hybridization assays.

Recommended Aminoallyl-dUTP-Cy5/dTTP ratio for PCR and Nick Translation: 20-30% Aminoallyl-dUTP-Cy5 / 80-70% dTTP.

Note: The optimal final concentration of the Dye-labeled dUTP may very depending on the application and assay conditions. For optimal product yields and high incorporation rates an individual optimization of the Dye-labeled-dUTP/dTTP ratio is recommended.

Structure



Cy5 excitation and emission spectra.

Spectroscopic data

Excitation maximum: λ_{Ex} = 649 nm

Emission maximum: λ_{Em} = 670 nm

Extinction coefficient: ϵ_{max} = 250,000 $\text{cm}^{-1} \text{M}^{-1}$

$CF_{260} = \epsilon_{260} / \epsilon_{max} = 0.05$

(Tris-HCl, pH 7.5)

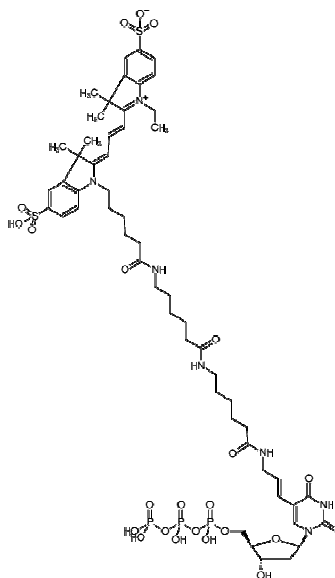
Cy5-dUTP, the dye is attached via an optimized linker to aminoallyl-dUTP.

Specifications

1 mM clear aqueous solution of 5-(3-aminoallyl)-2'-deoxy-uridine-5'-triphosphate labeled with Cy5, triethylammonium salt in 10 mM Tris-HCl (pH 7.5); Concentration: 1 mM – 1.1 mM
Purity: >95% (as determined by HPLC)

Molecular Formula: $C_{57}H_{80}N_7O_{23}P_3S_2$ (free acid)
Molecular Weight: 1388.33 g/mol (free acid)

Molecular Structure:



Storage and Stability

Store at -20°C in the dark. Short term exposure (up to 1 week cumulative) to ambient temperature possible. Avoid frequent thawing and freezing. Protect fluorescent labeled dUTP from light and carry out experimental procedures in low light conditions.

Labeling Protocol

Recommended PCR assay

20 μl PCR labeling assay

| Component | Stock conc. | Amount | Final conc. |
|---|------------------------|-----------------------------------|--------------------------------|
| High yield buffer without MgCl_2 | 10x | 2 μl | 1x |
| MgCl_2 stock solution | 25 mM | 1.6 μl | 2 mM |
| dATP | 1 mM | 2 μl | 100 μM |
| dCTP | 1 mM | 2 μl | 100 μM |
| dGTP | 1 mM | 2 μl | 100 μM |
| dTTP | 1 mM | 0.5-1 μl | 50 μM |
| Cy5-dUTP | 1 mM | 0.5-1 μl ¹⁾ | 50 μM ¹⁾ |
| forward Primer | 10 μM | 1 μl | 500 nM |
| reverse Primer | 10 μM | 1 μl | 500 nM |
| Template DNA | | 0.1-10 ng | 5-500 pg/ μl |
| Taq Pol | 5 units/ μl | 0.2 μl (1 unit) | 0.05 units/ μl |
| PCR grade H_2O | | Fill up to 20 μl | |

1) The optimal final concentration of the labeled nucleotide may vary depending on the application. Recommended ratio of Cy5-dUTP/dTTP is given above but has to be adjusted individually for optimal incorporation rates.

Recommended cycling conditions

| | | | |
|--------------------------|---------|--------|--------|
| Initial denaturation | 94°C | 2 min | 1x |
| Denaturation | 94°C | 30 sec | 25-30x |
| Annealing ¹⁾ | 50-60°C | 30 sec | |
| Elongation ²⁾ | 72°C | 1 min | |
| Final elongation | 72°C | 5 min | 1x |

1. The annealing temperature depends on the melting temperature of the primers used.
2. The elongation time depends on the length of the fragments to be amplified. A time of 2 min/kbp is recommended.

For optimal amplification results and high incorporation rates an individual optimization of the recommended PCR assay and cycling conditions may be necessary for each new primer-template pair.

Recommended NT assay

Sample Material can be supercoiled or linearized plasmid DNA, cosmid or BAC DNA, whole or partial chromosomes or purified PCR products.

Prepare the following reaction mixture in a sterile vial.

20 µl nick translation labeling assay

| amount | final conc. | component |
|------------------|-------------|---------------------|
| 2 µl | 1x | 10x Reaction buffer |
| 1 µl | 50 µM | dATP (1 mM) |
| 1 µl | 50 µM | dCTP (1 mM) |
| 1 µl | 50 µM | dGTP (1 mM) |
| 0.5 µl | 25 µM | dTTP (1 mM) |
| 0.5 µl | 25 µM | Cy5-dUTP (1 mM) |
| 1-1.5 µg | 50-75 ng/µl | Template DNA |
| | 0.2 u/µl | DNA Polymerase I |
| | 0.002 u/µl | DNase I |
| fill up to 20 µl | | PCR-grade water |

1. Vortex the mix gently to assure homogeneity and centrifuge briefly to collect the reaction mixture at the bottom of the tube.
2. Place the tube in a precooled thermomixer at 15°C. The incubation time strongly depends on the Polymerase I / DNase I activities. A well balanced enzyme ratio is required to generate labeled fragments in the desired size range. An individual optimization of the enzyme concentrations is recommended to generate DNA fragments in the range between 200 and 500 bp at an incubation time of 90 minutes.
3. To control the length of the fragments load 2 µl of the assay on an agarose gel. Place the reaction tube at -20°C while running the gel.
4. To get smaller fragments add again Polymerase I and Dnase I and extend the incubation at 15°C.
5. For final stopping the reaction add 5 µl EDTA (0.5 M, pH 8). Proceed to purification of the probe or store at -20°C.

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

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