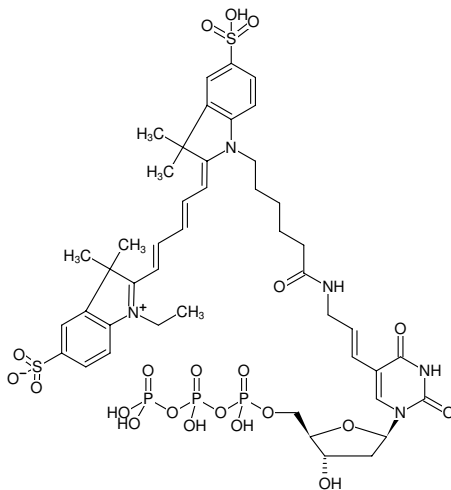




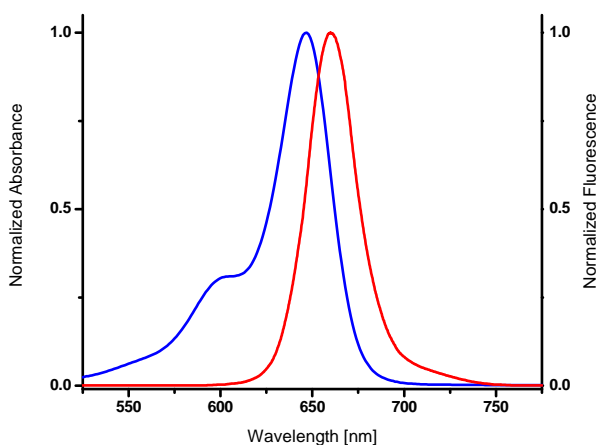
Cy5 NT Labeling Kit

Red fluorescent DNA labeling by nick translation

Cat. No.	Amount
PP-305S-CY5	10 reactions
PP-305L-CY5	50 reactions



Structural formula of Cy5 NT Labeling Kit



excitation and emission spectrum of Cy5

For general laboratory use.

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles, store dark

Shelf Life: 12 months

Spectroscopic Properties: λ_{exc} 649 nm, λ_{em} 670 nm, ϵ 250.0 L mol⁻¹ cm⁻¹ (Tris-HCl pH 7.5)

Description:

Cy5 Nick Translation Labeling Kit contains all reagents (except template and materials for purification of the probe) required for nick translation labeling providing a highly efficient, easy-to-perform and rapid labeling technology.

The kit is recommended for direct enzymatic labeling of DNA. The Cy5 NT labeling mix contains specially optimized dUTP-Cy5 for incorporation into DNA by nick translation using DNA Polymerase I. 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.

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 Polymerase I incorporates labeled dUTP instead of dTTP. The well balanced polymerase / nuclease activities of the enzyme mix ensure the generation of highly labeled double stranded DNA fragments.

The resultant DNA is suited for application in FISH, microarray gene expression profiling and other nucleic acid hybridization assays.

Protect fluorescent labeled dUTP from light and carry out experimental procedures in low light conditions.

Content:

Enzyme mix (red cap)

2 units/μl polymerase I, 0.02 units/μl Dnase I in storage buffer

NT labeling buffer (green cap)

10x conc.

Cy5 NT labeling mix (purple cap)

0.5 mM dATP, 0.5 mM dCTP, 0.5 mM dGTP, 0.25 mM dTTP, 0.25 mM dUTP-Cy5, pH 7.5

Stop buffer (yellow cap)

0.5 M EDTA, pH 8.0



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PCR-grade water (white cap)

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 μ l Nick translation labeling assay

fill up to 20 μ l	PCR-grade water	white cap
2 μ l	10x NT labeling buffer	green cap
2 μ l	Cy5 NT labeling mix	purple cap
1-1.5 μ g	template DNA	-
2 μ l	Enzyme mix	red cap

- Vortex the mix gently to assure homogeneity and centrifuge briefly to collect the reaction mixture at the bottom of the tube.
- Place the tube in a precooled thermomixer at 15 °C. An incubation of 90 min is recommended to generate DNA fragments in a size range between 200 and 500 bp.
- To control the length of the fragments load 2 μ l of the assay on an agarose gel. Place the reaction tube at -20 °C while running the gel.
- To get smaller fragments add additional 2 μ l of the Enzyme mix and extend the incubation at 15 °C.
- For final stopping the reaction add 5 μ l of Stop buffer (yellow cap). Proceed to purification or store at -20 °C.

Purification of the probe:

To remove unincorporated nucleotides from the reaction mixture prior to its use in subsequent experiments one of the following procedures is recommended:

1. *Purification by silica-gel membrane adsorption - PCR Purification Kit, Cat.-No. PP-201*

The Jena Bioscience PCR Purification Kit provides a simple and efficient way to purify DNA fragments larger than 100 bp. The preparation is based on a silica-membrane technology for binding DNA in high-salt and elution in low-salt buffer. Please refer to the instruction manual.

2. *Purification by Isopropanol precipitation*

Add 1 μ l glycogene (2 mg/ml), 2 μ l sodium acetate (3 M) and 14 μ l isopropanol to the reaction mixture and mix well but gently. Incubate on RT for 15 min and spin down at maximum speed at 4 °C for 30 min. Discard the supernatant and wash 2x with 70 % ethanol (spin down at maximum speed for 5 min).

3. *Purification by Centrifugal Filter Units*

Unincorporated nucleotides can be removed by centrifugation using centrifugal filter units. Select the filter unit by its cut-off for DNA fragments and follow the manufacturer's instructions.

Incorporation rate of the fluorophore:

The efficiency of DNA labeling can be estimated by calculating the ratio of incorporated fluorophores to the number of bases in the fragment (dye / base).

1. *Measurement of the optical density:* Measure the absorbance of the labeled DNA fragment at 260 nm (A_{260}) and at the excitation maximum (λ_{exc}) for the dye (A_{dye}).

2. *Correction of the A_{260} reading:* To obtain an accurate absorbance measurement for the nucleic acid, the contribution of the dye at 260 nm has to be corrected. Use the following equation:

$$A_{base} = A_{260} - (A_{dye} \times CF_{260})$$

Correction Factor for Cy5: $CF_{260} = 0.05$

3. *Calculation of the labeling rate:* The dye to base ratio is given by:

$$\text{dye / base} = (A_{dye} \times \epsilon_{base}) / (A_{base} \times \epsilon_{dye})$$

Extinction coefficients:

$$\text{Cy5: } \epsilon_{dye} = 250,000 \text{ cm}^{-1} \text{ M}^{-1}$$

$$\text{dsDNA: } \epsilon_{base} = 6,600 \text{ cm}^{-1} \text{ M}^{-1}$$

$$\text{ssDNA: } \epsilon_{base} = 8,900 \text{ cm}^{-1} \text{ M}^{-1}$$

$$\text{oligonucleotide: } \epsilon_{base} = 10,000 \text{ cm}^{-1} \text{ M}^{-1}$$

Example: A dye to base ratio of 0.05 corresponds to an incorporation of 10 dye-dUTP nucleotides into a DNA fragment containing 200 nucleotides or, respectively, into a 100 bp PCR fragment. If an equal distribution of dATP, dCTP, dGTP and dTTP in the DNA fragment can be assumed, 10 of the 50 existing dTTPs have been substituted by dye-dUTP resulting in a labeling rate of 20 %.

Related Products:

Aminoallyl-dUTP-Cy5, #NU-803-Cy5