コスモ・バイオ株式会社

Data Sheet

Jena Bioscience

Atto488 Nick Translation Labeling Kit

Kit for DNA labeling

DNA labeling by Nick Translation

Cat.-No.	Amount
PP-305S-488	10 reactions
PP-305L-488	50 reactions

For *in vitro* use only
Quality guaranteed for 12 months
Store at -20°C, avoid frequent thawing and freezing
NT labeling mix must be stored in the dark

Description

Atto488 Nick Translation Labeling Kit contains all reagents [1)] 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 Atto488 NT labeling mix contains specially optimized Atto488-dUTP 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 activitiy 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.

Enzyme mix (red cap)
2.5 units/µl Polymerase I, 0.01 units/µl DNase I
in storage buffer

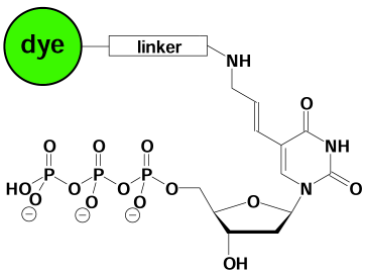
10x NT labeling buffer (green cap)
10x concentration

Atto488 NT labeling mix (purple cap)
0.5 mM dATP, 0.5 mM dCTP, 0.5 mM dGTP, 0.25 mM dTTP, 0.25 mM Atto488-dUTP, pH 7.5

Stop buffer (yellow cap)
0.5 M EDTA, pH 8.0

PCR-grade water (white cap)

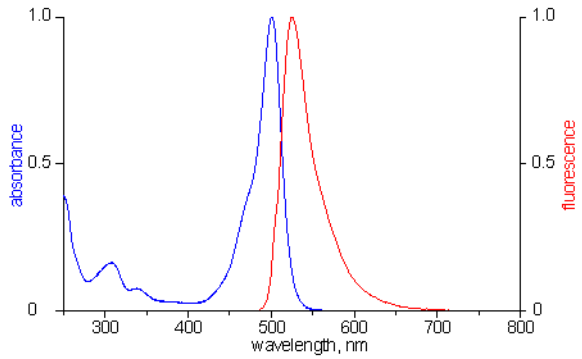
Structure



Atto488-dUTP, the dye is attached via an optimized linker to aminoallyl-dUTP

[1)] except template and materials for purification of the probe

Spectroscopic data
Excitation maximum: λ_{Ex} = 501 nm
Emission maximum: λ_{Em} = 523 nm
Extinction coefficient: ε_{max} = 90,000 cm^{-1} M^{-1}



Atto488 excitation and emission spectra

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		
2 µl	10x NT labeling buffer	green cap
2 µl	Atto488 NT labeling mix	purple cap
1-1.5 µg	Template DNA	
2 µl	10x Enzyme mix	red cap
Fill up to 20 µl	PCR-grade water	white cap

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. An incubation of 90 min is recommended to generate DNA fragments in a size range between 200 and 500 bp.
3. To control the length of the fragments load 2µl of the assay on an agarose gel. Place the reaction tube at -20°C while running the gel.
4. To get smaller fragments add additional 2 µl of the Enzyme mix and extend the incubation at 15°C.
5. For final stopping the reaction add 5µl 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 1µl glycogene (2 mg/ml), 2 µl sodium acetate (3 M) and 14 µl 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_{Ex}) 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 Atto488: CF_{260} = 0.25
3. *Calculation of the incorporation rate:* The dye to base ratio is given by:

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

   Extinction coefficients:
   Atto488: ε_{dye} = 90,000 cm^{-1} M^{-1}
   dsDNA: ε_{base} = 6,600 cm^{-1} M^{-1}
   ssDNA: ε_{base} = 8,900 cm^{-1} M^{-1}
   oligonucleotide: ε_{base} = 10,000 cm^{-1} M^{-1}

Example: A dye to base ratio of 0.05 corresponds to an incorporation of 5 dye-dUTP nucleotides into a 100 bp DNA fragment. This gives a substitution rate of 20% related to the total number of dTTPs if an equal distribution of dATP, dCTP, dGTP and dTTP in the fragment can be assumed.