

Atto425-dUTP-NT

Fluorescent labeled aminoallyl-dUTP

DNA labeling by Nick Translation

Cat.-No.	Amount	Conc.
PP-306S-425	10 µl	1 mM
PP-306L-425	50 µl	1 mM

For *in vitro* use only

Quality guaranteed for 12 months

Store at -20°C in the dark

Avoid frequent thawing and freezing

Description

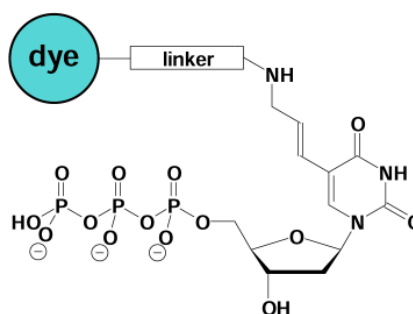
Atto425-dUTP-NT is recommended for direct enzymatic labeling of DNA. The dye-dUTP is specially optimized for incorporation into DNA by nick translation using DNA Polymerase I and DNase I. The excellent stability and quantum yield of the fluorophore combined with a high incorporation rate of the dye-dUTP 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 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 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.

Structure



Atto425-dUTP, the dye is attached via an optimized linker to aminoallyl-dUTP

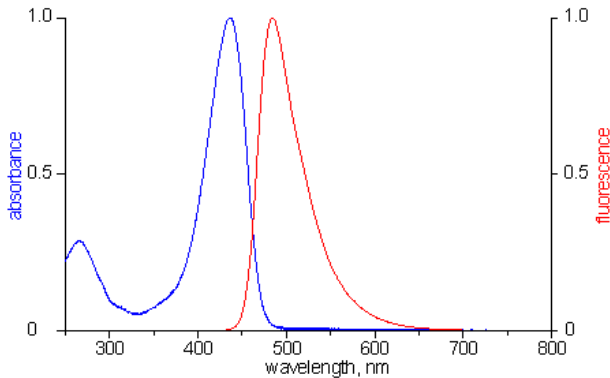
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1 mM aa-dUTP (5-(3-aminoallyl)-2'-deoxy-uridine-5'-triphosphate) labeled with Atto425, triethylammonium salt, pH 7.5, purity >95%

Spectroscopic data

 Excitation maximum: $\lambda_{Ex} = 436 \text{ nm}$

 Emission maximum: $\lambda_{Em} = 484 \text{ nm}$

 Extinction coefficient: $\epsilon_{max} = 45,000 \text{ cm}^{-1} \text{ M}^{-1}$


Atto425 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		
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	Atto425-dUTP-NT (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.

Related products

Nick Translation Labeling Kits
 PCR Labeling Kits
 Fluorescent Labeled Aminoallyl-dUTP for PCR
 Standard PCR / Thermophilic Polymerases
 Deoxynucleotides (dNTPs)
 Primers and Oligonucleotides
 DNA Ladders

For detailed information please visit
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