

# Atto425-dUTP-PCR

## Fluorescent labeled aminoallyl-dUTP

 DNA labeling by PCR
 

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Cat.-No.	Amount	Conc.
PP-302S-425	10 $\mu$ l	1 mM
PP-302L-425	50 $\mu$ l	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

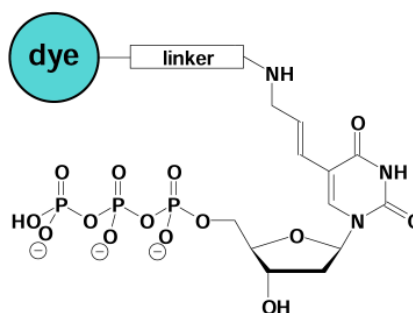
Atto425-dUTP-PCR is recommended for direct enzymatic labeling of DNA. The dye-dUTP is specially optimized for incorporation into DNA by PCR using Taq polymerase. The excellent stability and quantum yield of the fluorophore combined with a high incorporation rate of the dye-dUTP complex makes it the ideal choice for a broad range of fluorescence applications.

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 the 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.

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

### Atto425-dUTP-PCR

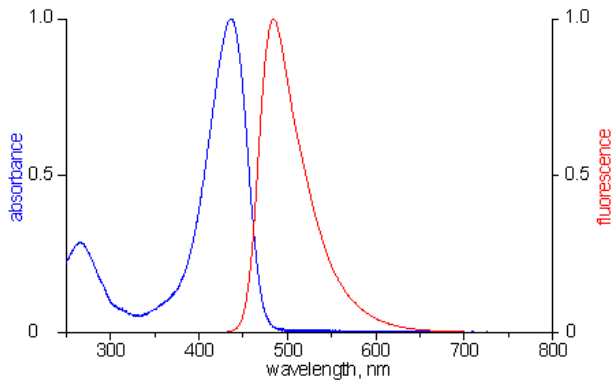
1 mM 5-(3-aminoallyl)-2'-deoxy-uridine-5'-triphosphate labeled with Atto425, triethylammonium salt, pH 7.5, purity >95%

### Spectroscopic data

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

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

Extinction coefficient:  $\epsilon_{max} = 45,000$  cm<sup>-1</sup> M<sup>-1</sup>



Atto425 excitation and emission spectra

**Recommended cycling conditions**

Initial denaturation	94 °C	2 min	1x
Denaturation	94 °C	30 sec	25-30x
Annealing <sup>1)</sup>	50-60 °C	30 sec	
Elongation <sup>2)</sup>	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.

**Recommended PCR assay**

20 µl PCR labeling assay			
Component	Stock conc.	Amount	Final conc.
High yield buffer without MgCl <sub>2</sub> (Cat.-No. PCR-201)	10x	2 µl	1x
MgCl <sub>2</sub> 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	1.5 µl	75 µM
Atto425-dUTP-PCR	1 mM	0.5 µl <sup>1)</sup>	25 µM <sup>1)</sup>
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 (Cat.-No. PCR-201)	5 units/µl	0.2 µl (1 unit)	0.05 units/µl
PCR grade H <sub>2</sub> O		Fill up to 20 µl	

- 1) The optimal final concentration of the labeled nucleotide may vary depending on the application.

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.

**Related products**

Kits for DNA labeling  
 Standard PCR / Thermophilic Polymerases  
 Deoxynucleotides (dNTPs)  
 Primers and Oligonucleotides  
 DNA Ladders

For detailed information please visit  
[www.jenabioscience.com/pcr](http://www.jenabioscience.com/pcr)