

Atto488 PCR Labeling Kit

Kit for DNA labeling

DNA labeling by PCR

Cat.-No.	Amount
PP-301S-488	10 reactions
PP-301L-488	50 reactions

For *in vitro* use only

Quality guaranteed for 12 months

Store at -20°C, avoid frequent thawing and freezing

PCR labeling mix must be stored in the dark

Description

Atto488 PCR Labeling Kit contains all reagents¹⁾ required for PCR labeling providing a highly efficient, easy-to-perform and rapid labeling technology.

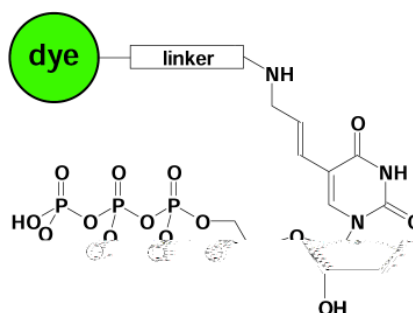
The kit is recommended for direct enzymatic labeling of DNA. The Atto488 PCR labeling mix contains specially optimized Atto488-dUTP for incorporation into DNA by PCR using Taq polymerase. The excellent stability and quantum yield of the fluorophore combined with a high incorporation rate of the dye-dUTP 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. When dTTP is partially substituted by dye-dUTP the extension of the annealed primers with Taq polymerase generates fluorescent labeled double-stranded DNA.

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



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

Spectroscopic data

Excitation maximum: $\lambda_{Ex} = 501 \text{ nm}$

Emission maximum: $\lambda_{Em} = 523 \text{ nm}$

Extinction coefficient: $\epsilon_{max} = 90,000 \text{ cm}^{-1} \text{ M}^{-1}$

Taq Pol (red cap)

2 unit/ μl Taq Polymerase in storage buffer

10x PCR labeling buffer (green cap)

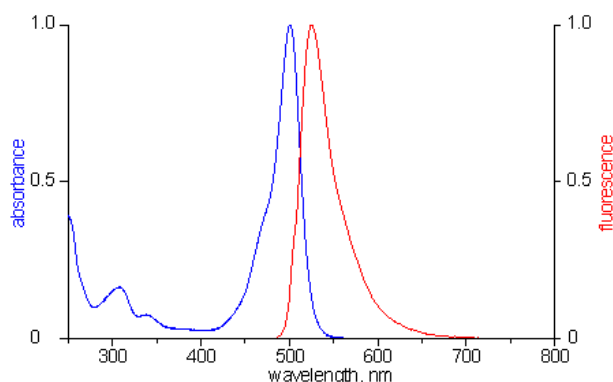
10x concentration

Atto488 PCR labeling mix (purple cap)

1 mM dATP, 1 mM dCTP, 1 mM dGTP, 0.5 mM dTTP, 0.5 mM Atto488-dUTP, pH 7.5

PCR grade water (white cap)

¹⁾ except primer, template and materials for purification of the probe



Atto488 excitation and emission spectra

Recommended PCR assay

Prepare the following reaction mixture in a sterile vial, adding the enzyme last.

20 µl PCR labeling assay		
to 20 µl final volume	PCR grade H ₂ O	white cap
2 µl	10x PCR labeling buffer	green cap
2 µl	Atto488 PCR labeling mix	purple cap
1 µl	forward Primer (10 µM)	
1 µl	reverse Primer (10 µM)	
0.1-10 ng	Template DNA	
0.5 µl (1 unit)	Taq Pol	red cap

Vortex the mix gently to assure homogeneity and centrifuge briefly to collect the reaction at the bottom of the tube. Place the tube in a thermocycler.

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 primers used.
- 2) The elongation time depends on the length of 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.

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 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:

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

Extinction coefficients:

Atto488: $\epsilon_{dye} = 90,000 \text{ cm}^{-1} \text{ M}^{-1}$

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

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

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 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.

For further information and related products, please visit us at: www.jenabioscience.com/pcr