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Atto 488 Protein Labelling Kit
Product No. 92313

Introduction

The Atto 488 Protein Labelling Kit provides a fast labelling of proteins as well an easy purification of the resulting conjugate. The succinimidyl ester group of the dye enables an effective reaction with primary amino groups of the proteins. The kit contains everything for 5 labellings and purification procedures. It is optimized for 1 mg protein per reaction. A final molar dye / protein (D/P) ratio between 2 – 9 can be expected, depending on the kind of protein.[1)]

Kit components

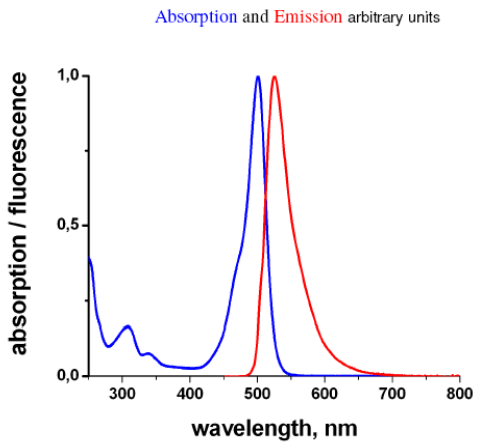
A) 5 vials Atto 488 reactive dye, each containing 0,24 mg dye
(optimized for 1 mg protein); product nr. 41698
B) Sodium bicarbonate buffer solution, pH 8,4, 10 ml, product nr. 88975
C) Phosphate buffer solution, pH 7,5, 100 ml, product nr. 76847
D) Protein purification set (containing 5 Spin Columns, 5 washing tubes, 5 sample collection tubes), product nr. 18932
E) Application note

Storage

Store the kit at 4°C protected from moisture. Protect the reactive dye from light.

Label characteristics

Atto 488 NHS ester (product nr. 41698)
$\lambda_{abs\ max}$ = 498 nm (in 0,1 M phosphate buffer solution pH 7,0)
$\lambda_{em\ max}$ = 520 nm (in 0,1 M phosphate buffer solution pH 7,0)
ε= 105 000 $cm^{-1}M^{-1}$



Labelling protocol

1. Dissolve 2 – 10 mg / ml protein in sodium bicarbonate buffer solution (B) (pH 8,4). Protein concentration < 2 mg / ml will decrease labelling efficiency of labelling reaction. Protein solution has to be free of amino group-containing substances (eg. Tris buffer solution or ammonium ions). Azide with concentration < 3 mM will not affect labelling efficiency.
2. Dissolve the reactive dye (A) in 20 µl sodium bicarbonate buffer (B).

3. Transfer the protein solution to the vial of reactive dye.
4. Incubate the reaction mixture for 2 hours while gently stirring and protecting the vial from light. It is recommend to prepare the dye solution immediately before starting the coupling reaction. Due to the different reactivity of proteins the degree of labelling might vary between 2 – 9. The incubation time can be prolonged up to 12 h at 4°C.

Separation of conjugates from free dye

The purification set (D, spin column, wash tube and collection tube) is designed for 100 µL volume capacity containing 1 mg protein (e.g. IgG). Regenerate gel inside column before adding your sample:

1. Remove top cap of the column
2. Add 650 µl phosphate buffer (C)
3. Vortex column (replace top cap before) for a few seconds
4. Allow hydration for at least 30 min.
5. Remove top and bottom cap, place column into a wash tube and put both together into the rotor of your centrifuge
6. Spin column for 2 min. (750 x g)
7. Replace wash tube by one collection tube
8. Discard the wash tube containing excess fluid
9. Transfer your sample (max. 100 µl) to the top of the gel
10. Spin the column and collection tube for 2 min. (750 x g)
11. Remove column from the collection tube; purified protein is located in the collection tube

Determination of dye/ protein (D/P) ratio

After a successful separation of the free dye from the labelled protein, the dye to protein ratio (D/P) of the conjugate can be calculated:
Measure the absorbance of the conjugates at 280 nm and 498 nm. Before measuring, the conjugate should be diluted such that the maximum absorbance measured is between 0.5 and 1.0 A.U..

The D/P molar ratio can be calculated as follows:

$$D/P = \frac{A_{498} \times \varepsilon_{Protein}}{\left[A_{280} - (A_{498} \times 0.101)\right] \times 105{,}000}$$

A_{498}= absorbance at 498nm measured in a cuvette with a pathlength of 1 cm
A_{280} = absorbance at 280 nm measured in a cuvette with a pathlength of 1 cm
$\varepsilon_{Protein}$ = molar extinction coefficient of the protein 280 nm [$cm^{-1}M^{-1}$]. IgG usually have ε of 203,000.
105,000 = molar extinction coefficient (ε_{dye}) of the Atto 488 dye at 498 nm [$cm^{-1}M^{-1}$]
0.101 = correction factor due to the fluorophore's absorbance at 280 nm

The final protein concentration can be calculated as follows:

$$c_{Protein}\ (mg/ml) = \frac{A_{280} - (0.101 \times A_{498})}{\varepsilon_{Protein}} \times MW_{Protein} \times Dilution\ \ factor$$

$MW_{Protein}$ = Molecular weight of the protein (for IgG 150,000 g / mol)
Dilution factor: Dilution of the labelled conjugate prior to measurement by spectrophotometer.

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Handling of labelled conjugates

Labelled conjugates should be stored at 4°C and protected from light. To improve stability sodium azide (0,01 %) may be added. The conjugates should be stable for several month. For extended storage solution should be kept at – 20°C. Avoid repeated freeze-thaw cycles of conjugates. In order to prevent denaturation of the conjugates add bovine serum albumin (BSA) to a final concentration of 1 – 10 mg / ml or use our BioStab biomolecule storage solution (Fluka 92889).

Reference

E. Harlow, D. Lane, Using *Antibodies, A Laboratory Manuel*, Cold Spring Harbor Laboratory Press, New York, **1999**