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DATASHEET

Atto565-Actin

Atto565-Actin

From rabbit skeletal muscle
(α-skeletal muscle actin)

For Use in Research Only.
Not for Use in Diagnostic Processes.

Quantity: 100µg
Cat.#: 8162-01
Quantity: 5x100µg
Cat.#: 8162-02

Product Description

G-actin from rabbit skeletal muscle is a single chain polypeptide with a molecular weight of 42kD consisting of 375 amino acids. Atto565-Actin is chemically modified G-actin with an Atto 565 fluorescent group covalently bound to lysine residues. Atto565-Actin is >99% pure according to scanning densitometry from Coomassie Blue G-250 stained PAA-Gels and possesses the polymerization properties of native G-actin. The DOL of Atto565-Actin is 0.5.

Atto565-Actin is supplied as a lyophilized powder containing 2mM Tris-Cl pH 8.2, 0.4mM ATP and 0.1mM DTT, 1mM NaN_3, 0.2mM $CaCl_2$ and 0.5% disaccharides when reconstituted with H_2O to a 1mg/ml (23.8µM) solution.

For dilution of G-actin or exchange of ligand buffer into actin compatible buffer, MonoMix (Cat no.: 5100-0*) may be used. In the absence of nucleators, the polymerization of G-actin can be initiated by PolyMix (Cat no.: 5000-0*).

Product Handling

Preparation of a working stock

Example: Add 100µl of H_2O to the tube containing 100µg Atto565-Actin to obtain a working stock of 1mg/ml (23.8µM) and dissolve by pipetting up and down. Allow the G-actin solution to rehydrate for 2 minutes at room temperature being protected from light. Further dissolve the powder by pipette to obtain a homogeneous, pinkish Atto565-Actin solution. Leave at RT for another 2 min for rehydration. The final concentration of G-actin should not exceed 3mg/ml. For standard applications the Atto565-Actin working stock is now ready to use.

For critical assays the working stock should be applied to a gel filtration column or dialyzed (100vol. of MonoMix, O/N) followed by high-speed centrifugation. For less critical assays the actin working stock may be pre-spun (15.000rpm, 10min).

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Product Storage and Stability

For best product performance Atto565-Actin is stored as supplied at –70° until used. Once dissolved Atto565-Actin is kept on ice and protected from light. Under these conditions the product will be stable for 5 days. Avoid refreezing of solubilised Atto565-Actin.

Specifications of Atto565-Actin

Esterification of α-skeletal muscle actin with ATTO565 results in a chemically modified G-actin carrying a fluorescent dye with a net electrical charge of 0. The fluorescence is excited most efficiently at 592nm.

Determining the Degree of Labeling (DOL)

1. Protein Concentration

Determination of the protein concentration by UV-VIS of nucleotide binding proteins is affected by the presence of nucleotides in buffer and protein. Nucleotides strongly absorb at 280nm. Minor variations in buffer composition result in erroneous measurements. For a correct UV-VIS measurement actin should be measured at 290nm (ε =26,600 $M^{-1}cm^{-1}$), after exchange of buffer against the reference buffer.

2. Degree of Labelling

The degree of labeling (DOL or dye/protein ratio) is usually determined by absorption spectroscopy making use of the Lambert-Beer law: Absorbance (A) = extinction coefficient (ε) × molar concentration × path length (d). Simply measure the UV-VIS spectrum of the conjugate in solution in a quartz cuvette. Dilute the solution, if necessary to measure within the linear range.

$$DOL = \frac{A_{max} / \varepsilon_{max}}{A_{prot} / \varepsilon_{prot}} = \frac{A_{max} \cdot \varepsilon_{prot}}{A_{280} - (A_{max} \cdot CF_{280}) \cdot \varepsilon_{max}}$$

A_{max} = maximal absorbance at 563nm of the dye measured in a cuvette with a pathlength of 1 cm).

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ε_{prot} = 26,600 $M^{-1}cm^{-1}$ (molar decadic extinction coefficient at the longest-wavelength absorption maximum).

ε_{max} = 120,000 $M^{-1}cm^{-1}$ (molar decadic extinction coefficient at the longest-wavelength absorption maximum).

CF_{290} = 0.20 ($CF_{280} = \varepsilon_{280}/\varepsilon_{max}$. Correction factor to calculate the degree of labeling (DOL) of dye-protein conjugates).

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