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DATASHEET

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

ATTO565-Actin for TIRFM

From porcine brain
(β/γ-non-muscle actin)

Quantity: 2x50µg
Cat.#: 8356-03

Product Description

G-actin from porcine brain is a mixture of β/γ non-muscle actin isoforms. Both isoforms are single chain polypeptides with a molecular mass of 42kD consisting of 375 amino acids. ATTO565-Actin (λex 563nm, λem 592nm) is chemically modified G-actin by coupling of NHS-ATTO565 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 0.3 and thus ready-to-use for TIRF microscopy. ATTO565-Actin is supplied as a lyophilized powder containing 2mM Tris-Cl pH 8.2, 1mM ATP, 0.1mM DTT, 0.2mM $CaCl_2$ and 0.6% disaccharides when reconstituted with H_2O to a 1mg/ml 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 50µl H_2O to a vial with 50µg ATTO565-Actin to obtain a working stock of 1mg/ml (23µM) and vortex for 30secs. The final concentration of G-actin should not exceed 3mg/ml. Allow the G-actin solution to rehydrate for 5min at room temperature, dissolve the pellet by pipetting several times up and down to obtain a homogeneous, yellowish ATTO565-Actin solution. Leave to fully rehydrate for another 2 min, vortex for 30secs and spin 1min, 15.000xg. For standard applications, the ATTO565-Actin working stock is ready to use. For critical assays the working stock should at least be dialyzed (100vol. of MonoMix, O/N) followed by high-speed centrifugation. For less critical assays the actin working stock may be filtered (0.2-0.45µm) or pre-spun (15.000rpm, 10min).

Product Storage and Stability

For best product performance ATTO565-Actin is stored as supplied at –70° for six months. Once dissolved, ATTO565-Actin is kept protected from light and on ice. Under these conditions the monomeric actin conjugate

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is stable for up to 5 days and for 2 weeks in the polymerized state. Avoid refreezing of solubilised, monomeric ATTO565-Actin and do not refreeze F-actin.

Specifications of ATTO565-Actin

The careful esterification of non-muscle actin with ATTO565 (λex 563nm, λem 592nm) results in a chemically modified G-actin. Covalent conjugation of the fluorescent dye results in a net electrical charge of -1 and increases the molecular mass of actin to be ~43kD. The absorption maximum is at 563nm and the emission maximum at 592nm. ATTO565-actin possesses the polymerization properties of native actin as determined by viscometry and analytical high-speed cosedimentation.

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.

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

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

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} = 0.08 ($CF_{280} = \varepsilon_{280}/\varepsilon_{max}$. Correction factor to calculate the degree of labeling (DOL) of dye-protein conjugates).

Related products

FluMaXx The oxygen scavenging system for single fluorescent molecule imaging
Cat. #: 5161-01

ATTO565-Actin α–skeletal muscle actin, rabbit
Cat. #: 8162-01 2x100µg
Cat. #: 8162-02 5x100µg

ATTO647-Actin α–skeletal muscle actin, rabbit
Cat. #: 8158-01 2x100µg
Cat. #: 8158-02 5x100µg

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