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# DATASHEET

## Atto488-Actin

From rabbit skeletal muscle
(α-skeletal muscle actin)

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

Quantity: 1x100µg
Cat.#: 8153-01
Quantity: 5x100µg
Cat.#: 8153-02

### Product Description

G-actin from rabbit skeletal muscle is a single chain polypeptide with a molecular mass of 42kD consisting of 375 amino acids. Atto488-Actin is chemically modified G-actin by coupling of NHS-Atto 488 to lysine residues. The protein/dye molar ratio of Atto488-Actin is 1:0.90. Atto488-Actin is supplied as a lyophilized powder containing 2mM Tris-Cl pH 8.2, 0.4mM ATP and 0.1mM DTT, 0.2mM $CaCl_2$ and 0.6% disaccharides when reconstituted with $H_2O$ to a 1mg/ml solution. The precise DOL is stated in the datasheet shipped with the product.

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 a vial with 100µg Atto488-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 Atto488-Actin solution. Leave to fully rehydrate for another 2 min, vortex for 30secs and spin 1min, 15.000xg. For standard applications the Atto488-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 Atto488-Actin is stored as supplied at –70° for six months. Once dissolved, Atto488-Actin is kept protected from light and on ice. Under these conditions the monomeric actin conjugate



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is stable for 5 days and for 2 weeks in the polymerized state. Avoid refreezing of solubilised, monomeric Atto488-Actin and do not refreeze F-actin.

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### Specifications of Atto488-Actin

Esterification of α-skeletal muscle actin with Atto488 (Abs.$_{max}$. 501 nm; Em.$_{max}$. 523 nm) 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 501 nm and the emission maximum at 523 nm. Atto488-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 ( $\varepsilon$=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 ($\varepsilon$) × 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 488 nm of the dye measured in a cuvette with a pathlength of 1 cm).

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



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$\varepsilon_{max}$ = 90,000 $M^{-1}cm^{-1}$ (molar decadic extinction coefficient at the longest-wavelength absorption maximum).

$CF_{290}$ = 0.1 ($CF_{280} = \varepsilon_{280}/\varepsilon_{max}$. Correction factor to calculate the degree of labeling (DOL) of dye-protein conjugates).





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