V. 1.0

Actin: HiLyte™ Fluor 555 Labeled
(Rabbit skeletal muscle, >99% pure)
Cat. # AR07-A
Lot # 131 Amount: 5 x 20 µg
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Material

Purified rabbit muscle actin has been modified to contain covalently linked HiLyte™555 fluorochrome at random surface lysine residues. An activated ester of HiLyte™555 is used to label the protein. The labeling stoichiometry has been determined to be 0.8 – 1.4 dyes per actin monomer. HiLyte™555 labeled rabbit muscle actin has an approximate molecular weight of 43 kDa, and is supplied as a lyophilized powder (dark pink color). AR06 has maximal absorbance at 550 nm and emission at 570 nm (Fig 1).

Applications

Application	Reference
Modeling *in vitro* bio membranes	1, 2
Molecular mechanisms underlying cytoskeletal mediated force/stress	3, 4, 5, 6
In vitro modeling of the	7
Study mechanisms of *in vivo* actin dynamics by labeling of free barbed ends of actin filaments	8, 9, 10,11
Study actin binding proteins	12,13, 14
Applications in functional nanodevices	15,16

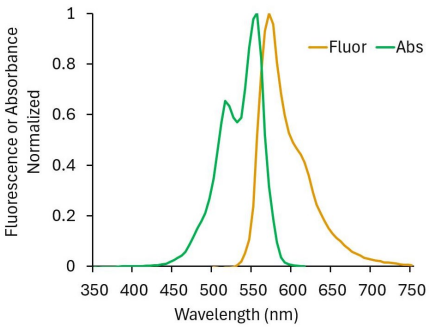
Storage and Reconstitution

Briefly centrifuge to collect the product at the bottom of the tube. The lyophilized protein is stable for 6 months when stored desiccated to <10% humidity at 4°C. The protein should be reconstituted to 10 mg/ml with 2 µl of nanopure water; it will then be in the following buffer: 5 mM Tris-HCl pH 8.0, 0.2 mM $CaCl_2$, 0.2 mM ATP, 5% (w/v) sucrose and 1% (w/v) dextran. The concentrated protein can then be snap frozen in liquid nitrogen and stored at -70°C where it is stable for 6 months. For working concentrations, further dilution of the protein should be made with General Actin Buffer (Cat. # BSA01) supplemented with 0.2 mM ATP (Cat. # BSA04) and 0.5 mM DTT. Fluorescent muscle actin is a labile protein and should be handled with care. Avoid repeated freeze-thaw cycles.

Purity

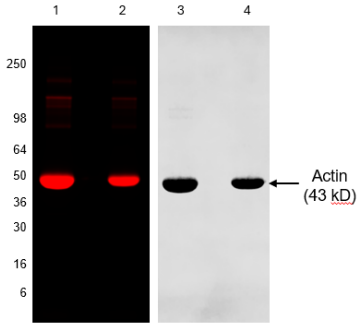
Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gel. HiLyte™555 labeled actin was found to be >99% pure (see Figure 2).

Figure 1: Absorbance & Fluorescence Scan for AR07



Legend-Fig. 1: AR07 was diluted with nanopure water and its absorbance (green line) and fluorescence (orange line) spectra were scanned between 350 and 750 nm. Fluorescent labeling stoichiometry was calculated to be 0.8-1.4 dyes per actin protein using the absorbance maximum for HiLyte™555 fluorescence at 540 nm and the Beer-Lambert law. The extinction coefficient of the dye is 150,000 $M^{-1}cm^{-1}$.

Figure 2: Actin HiLyte™555 Protein Purity Determination



Legend-Fig. 2: 20 µg (Lanes 1 & 3) and 10 µg (Lanes 2 & 4) of AR07 was analyzed by electrophoresis in a 4-20% SDS-PAGE system. A Licor Odessy gel analysis was performed 600nm (HiLyte™555, lanes 1 & 2) and at 700nm (Coomassie, lanes 3 & 4), Protein quantitation was determined with the Precision Red™ Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen.

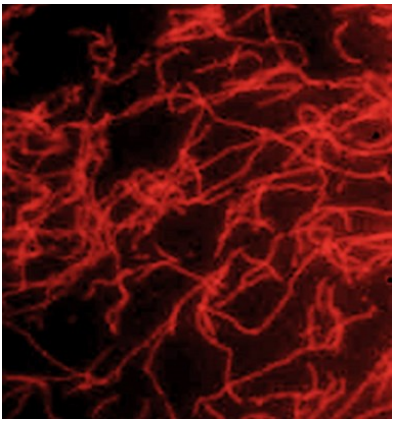
Quality Control: Polymerization spin down assay

The biological activity of HiLyte™555 actin can be determined by its ability to efficiently polymerize into filaments *in vitro* and separate from unpolymerized components in a spin down assay. Stringent quality control ensures that ≥90% of the labeled muscle actin can polymerize in the presence of polymerization buffer & ≤5% polymer is present in the absence of polymerization buffer.

***In vitro* polymerization of HiLyte™555 actin to create labeled actin filaments**

1. Resuspend HiLyte™555 actin muscle actin to 0.4 mg/ml with General Actin Buffer supplemented with 0.2 mM ATP and 1 mM DTT. Add 1/10th the volume of Polymerization Buffer supplemented with 1 mM DTT and incubate at room temperature for 1 h.
2. Dilute the polymerized actin filaments 100 fold in 1x Polymerization Buffer containing 70 nM phalloidin and spot 1 µl into a drop of anti-fade solution on a microscope slide.
3. Place a coverslip over the drop and remove excess liquid with a tissue.
4. Observe HiLyte™555 labeled actin filaments with a fluorescent microscope.
5. A typical fluorescent image is shows in Figure 3.

Figure 3: Fluorescent image of HiLyte™555 actin filaments



HiLyte™555 actin muscle actin was polymerized for 1 h, spotted onto a microscope slide and observed by epifluorescence microscopy equipped with a digital CCD camera and 100x objective. Fluorescent filaments were observed using a TRITC filter set Ex: 525±15 / Em: 595±20

Advice for Working with Muscle Actin

1. Monomer actin is unstable in the absence of ATP (0.2 mM), a divalent cation (0.2 mM $CaCl_2$ or 10 µM $MgCl_2$) and dithiothreitol (1 mM)
2. Monomer actin will polymerize at >20 mM K+, Na+, and in >0.2 mM Mg^{2+}.
3. Monomer actin will not polymerize at <2 mM K+, Na+, or in <0.05 mM Mg^{2+}.
4. Monomer actin is unstable below pH 6.5, or above pH 8.5.
5. Snap freeze actin in liquid nitrogen at 10 mg/ml to maintain high biological activity.

Application References

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