



Revised: April 15, 2020

Product Information: Fluorescence Labeled Phalloidin

| Compound | Storage information | Shelf Life |
|---|---|---|
| Fluorescent phalloidin, solvent-free lyophilized solid | Freeze upon receipt < -20 °C Protect from light and moisture | When stored as indicated, fluorescence labeled phalloidin is stable for at least 3 years. |
| For optical properties see Table 1 on page 2. | | |

Introduction:

Phalloidin is a phallotoxin isolated from *Amanita Phalloides*. It is a bicyclic peptide of about 800 daltons and 1.5 nm in diameter. Fluorescence labeled amino-phalloidin stains actin filaments (F-actin) with very high specificity, making it an extremely useful tool to visualize the skeleton of cells. In contrast to antibodies, the binding affinity of labeled phalloidin does not change notably for actin of different species or sources. Due to its relatively small size actin binding proteins like myosin, tropomyosin, vimentin, troponin etc. are still able to bind to previously with phalloidin stained actin. The contrast between labeled and unlabeled actin is extremely high. This is mainly due to negligible nonspecific staining making it the ideal probe for microscopy applications. Phalloidin conjugates are available for all ATTO-labels.

Storage and Handling:

ATTO-dye labeled phalloidin is supplied as solvent-free lyophilizate and should be stored at < -20 °C, desiccated and protected from light. When stored as indicated, the product is stable for at least three years.

For the preparation of stock solutions the **ATTO**-phalloidin conjugate (10 nmol unit) should be dissolved in 1 ml of methanol or water/methanol (see Table 1) to yield a concentration of 10 µM. Protect from light and store at 2 – 6 °C. Such solutions are stable for up to six months. For long-term storage you may divide the solution into aliquots and freeze at -20 °C.

Note: Depending on solvent quality the shelf-life of such solutions might be significantly reduced compared to the dye-conjugate in its solid form.

Labeling with ATTO-Phalloidin Conjugates:

Dissolve the vial content in 1 ml of methanol or water/methanol (see Table 1) to obtain a stock solution providing 300 units, thus one unit corresponds to 3.3 µl. One unit is generally sufficient material to stain e.g. one microscope slide of fixed cells. For staining dilute 3.3 µl of methanolic stock solution with 200 µl phosphate-buffered saline (PBS), pH 7.4 for each coverslip. It might also be advantageous to pre-equilibrate fixed cells with PBS containing 1 % bovine serum albumin (BSA) for 30 minutes prior to phalloidin staining. For a detailed sample preparation and staining procedure we refer to reference 1. However, one needs to keep in mind that experimental improvement might be eligible for method optimization.

References:

1. van de Linde S.; Heilemann M. ; Sauer M. et al., *Direct stochastic optical reconstruction microscopy with standard fluorescent probes*, Nature Protocols **6** (7), (2011), 991-1009.

Table 1: Properties of ATTO-dye labeled phalloidin:

| Dye | Order # | λ_{abs} | λ_{em} | ϵ_{max} | MW | M ⁺ | Solvent |
|-------------|-------------|------------------------|-----------------------|-------------------------|------|----------------|--------------------|
| ATTO 390 | AD 390-8 | 390 | 476 | 24000 | 1113 | 1113 | Methanol |
| ATTO 425 | AD 425-8 | 439 | 485 | 45000 | 1171 | 1172 | Methanol |
| ATTO 430LS | AD 430LS-8 | 436 | 545 | 32000 | 1359 | 1337 | Methanol |
| ATTO 465 | AD 465-8 | 453 | 506 | 75000 | 1179 | 1065 | Methanol |
| ATTO 488 | AD 488-8 | 500 | 520 | 90000 | 1473 | 1359 | Water/Methanol |
| ATTO 490LS | AD 490LS-8 | 495 | 658 | 40000 | 1466 | 1444 | Water/Methanol |
| ATTO 495 | AD 495-8 | 498 | 526 | 80000 | 1235 | 1122 | Methanol |
| ATTO Rho110 | AD Rho110-8 | 507 | 531 | 100000 | 1313 | 1199 | Methanol |
| ATTO 514 | AD 514-8 | 511 | 532 | 115000 | 1638 | 1523 | Water/Methanol 2:8 |
| ATTO 520 | AD 520-8 | 517 | 538 | 110000 | 1250 | 1136 | Methanol |
| ATTO 532 | AD 532-8 | 532 | 552 | 115000 | 1530 | 1530 | Methanol |
| ATTO Rho6G | AD Rho6G-8 | 533 | 557 | 115000 | 1398 | 1283 | Methanol |
| ATTO 540Q | AD 540Q-8 | 543 | | 105000 | 1443 | 1329 | Methanol |
| ATTO 542 | AD 542-8 | 542 | 562 | 120000 | 1798 | 1683 | Water/Methanol |
| ATTO 550 | AD 550-8 | 554 | 576 | 120000 | 1478 | 1363 | Methanol |
| ATTO 565 | AD 565-8 | 564 | 590 | 120000 | 1394 | 1280 | Methanol |
| ATTO Rho3B | AD Rho3B-8 | 566 | 589 | 120000 | 1426 | 1312 | Methanol |
| ATTO Rho11 | AD Rho11-8 | 572 | 595 | 120000 | 1450 | 1336 | Methanol |
| ATTO Rho12 | AD Rho12-8 | 577 | 600 | 120000 | 1530 | 1416 | Methanol |
| ATTO Thio12 | AD Thio12-8 | 582 | 607 | 110000 | 1386 | 1271 | Methanol |
| ATTO Rho101 | AD Rho101-8 | 587 | 609 | 120000 | 1474 | 1360 | Methanol |
| ATTO 580Q | AD 580Q-8 | 587 | | 110000 | 1579 | 1465 | Methanol |
| ATTO 590 | AD 590-8 | 593 | 622 | 120000 | 1475 | 1360 | Methanol |
| ATTO 594 | AD 594-8 | 603 | 626 | 120000 | 1688 | 1575 | Water/Methanol |
| ATTO Rho13 | AD Rho13-8 | 603 | 627 | 120000 | 1530 | 1416 | Methanol |
| ATTO 610 | AD 610-8 | 616 | 633 | 150000 | 1274 | 1161 | Methanol |
| ATTO 612Q | AD 612Q-8 | 615 | | 115000 | 1575 | 1461 | Methanol |
| ATTO 620 | AD 620-8 | 620 | 642 | 120000 | 1396 | 1282 | Methanol |
| ATTO Rho14 | AD Rho14-8 | 626 | 646 | 140000 | 1668 | 1552 | Methanol |
| ATTO 633 | AD 633-8 | 630 | 651 | 130000 | 1436 | 1321 | Methanol |
| ATTO 643 | AD 643-8 | 643 | 665 | 150000 | 1741 | 1627 | Water/Methanol |
| ATTO 647 | AD 647-8 | 647 | 667 | 120000 | 1477 | 1363 | Methanol |
| ATTO 647N | AD 647N-8 | 646 | 664 | 150000 | 1530 | 1415 | Methanol |
| ATTO 655 | AD 655-8 | 663 | 680 | 125000 | 1412 | 1297 | Methanol |
| ATTO Oxa12 | AD Oxa12-8 | 662 | 681 | 125000 | 1523 | 1409 | Methanol |
| ATTO 665 | AD 665-8 | 662 | 680 | 160000 | 1507 | 1392 | Methanol |
| ATTO 680 | AD 680-8 | 681 | 698 | 125000 | 1410 | 1295 | Methanol |
| ATTO 700 | AD 700-8 | 700 | 716 | 120000 | 1450 | 1335 | Methanol |
| ATTO 725 | AD 725-8 | 728 | 751 | 120000 | 1299 | 1185 | Methanol |
| ATTO 740 | AD 740-8 | 743 | 763 | 120000 | 1352 | 1237 | Methanol |
| ATTO MB2 | AD MB2-8 | 668 | | 100000 | 1239 | 1125 | Methanol |

λ_{abs} : longest wavelength absorption maximum in nm; λ_{em} : fluorescence maximum in nm; ϵ_{max} : molar decadic extinction coefficient at the longest-wavelength absorption maximum in M⁻¹ cm⁻¹; MW: molecular weight of the dye including counterions in g/mol; M⁺: molecular weight of dye cation (HPLC_MS acetonitrile/water 0.1 vol-% trifluoroacetic acid)

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