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.

Table 1: Properties of ATTO-dye labeled phalloidin:

Dye	λ abs	λ em	ε max	MW	M*	Solvent
ATTO 390	390	476	24000	1113	1113	Methanol
ATTO 425	439	485	45000	1171	1172	Methanol
ATTO 430LS	436	545	32000	1359	1337	Methanol
ATTO 465	453	506	75000	1179	1065	Methanol
ATTO 488	500	520	90000	1473	1359	Water/Methanol 1:1
ATTO 490LS	495	658	40000	1466	1444	Water/Methanol 1:1
ATTO 495	498	526	80000	1235	1122	Methanol
ATTO Rho110	507	531	100000	1313	1199	Methanol
ATTO 514	511	532	115000	1638	1523	Water/Methanol 1:1
ATTO 520	517	538	110000	1250	1136	Methanol
ATTO 532	532	552	115000	1530	1415	Methanol
ATTO Rho6G	533	557	115000	1398	1283	Methanol
ATTO 540Q	543		105000	1443	1329	Methanol
ATTO 542	542	562	120000	1798	1683	Water/Methanol 1:1
ATTO 550	554	576	120000	1478	1363	Methanol
ATTO 565	564	590	120000	1394	1280	Methanol
ATTO Rho3B	566	589	120000	1426	1312	Methanol
ATTO Rho11	572	595	120000	1450	1336	Methanol
ATTO Rho12	577	600	120000	1530	1416	Methanol
ATTO Thio12	582	607	110000	1386	1271	Methanol
ATTO Rho101	587	609	120000	1474	1360	Methanol
ATTO 580Q	587		110000	1579	1465	Methanol
ATTO 590	593	622	120000	1475	1360	Methanol
ATTO 594	603	626	120000	1688	1575	Water/Methanol 1:1
ATTO Rho13	603	627	120000	1530	1416	Methanol
ATTO 610	616	633	150000	1274	1161	Methanol
ATTO 612Q	615		115000	1575	1461	Methanol
ATTO 620	620	642	120000	1396	1282	Methanol
ATTO Rho14	626	646	140000	1668	1552	Methanol
ATTO 633	630	651	130000	1436	1321	Methanol
ATTO 643	643	665	150000	1741	1627	Water/Methanol 1:1
ATTO 647	647	667	120000	1477	1363	Methanol
ATTO 647N	646	664	150000	1530	1415	Methanol
ATTO 655	663	680	125000	1412	1297	Methanol
ATTO Oxa12	662	681	125000	1523	1409	Methanol
ATTO 665	662	680	160000	1507	1392	Methanol
ATTO 680	681	698	125000	1410	1295	Methanol
ATTO 700	700	716	120000	1450	1335	Methanol
ATTO 725	728	751	120000	1299	1185	Methanol
ATTO 740	743	763	120000	1352	1237	Methanol
ATTO MB2	668		100000	1239	1125	Methanol

λabs: longest wavelength absorption maximum in nm; λem: fluorescence maximum in nm; εmax: 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)

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.