Introduction:

Labels with an amine moiety can be used for coupling to carboxy groups via a mild in-situ activation or by direct coupling to succinimidyl esters, TFP-esters or mixed anhydrides.

Storage and Handling:

ATTO amino-modified labels are generally supplied as dry, crystalline solids 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 we recommend to dissolve the dye (1 mg unit) in 200 - 500 µl of dry DMSO or DMF - for ATTO MB2 use acetonitrile. Stock solutions should be stored at -20 ° C and protected from light.

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

Labeling with Amino-Modified ATTO-Labels:

The reactivity of an amine strongly depends on its basicity. All amino-modified ATTO-labels are aliphatic amines. The concentration of the reactive, free base (-NH2) of aliphatic amines below pH 8 is very low. Thus, the reaction kinetics of amine acylation by succinimidyl esters or other reagents is strongly pH dependent. Therefore, coupling reactions should be performed at pH 8.5 or higher. ATTO amino-modified labels are provided as ammonium salts. Due to the risk of hydrolysis of e.g. succinimidyl esters or anhydrides in aqueous solution it is, whenever possible, advantageous to work in anhydrous organic solvents (DMF or DMSO) and to add 1.5 - 2 eq. of N,N-diisopropylethylamine (Hünigs base), to ensure a sufficiently high concentration of free amine (-NH2) for the reaction to take place.

Table: Properties of amino-modified ATTO-labels:

Dye	MW	M+	λ abs	λ em	ε max	CF260	CF280
ATTO 390	500	386	390	476	24000	0.46	0.09
ATTO 425	558	444	439	485	45000	0.19	0.17
ATTO 465	565	338	453	506	75000	1.09	0.48
ATTO 488	860	632	500	520	90000	0.22	0.09
ATTO 495	625	395	498	526	80000	0.45	0.37
ATTO 514	1024	796	511	532	115000	0.21	0.07
ATTO 520	609	409	517	538	110000	0.16	0.20
ATTO 532	916	688	532	552	115000	0.2	0.09
ATTO Rho6G	784	556	533	557	115000	0.19	0.16
ATTO 550	864	636	554	576	120000	0.23	0.1
ATTO 565	781	553	564	590	120000	0.27	0.12
ATTO 590	917	689	593	622	120000	0.39	0.43
ATTO 594	1076	848	603	626	120000	0.22	0.5
ATTO 633	822	594	630	651	130000	0.04	0.05
ATTO 643	1106	878	643	665	150000	0.05	0.04
ATTO 647N	889	688	646	664	150000	0.04	0.03
ATTO 655	798	570	663	680	125000	0.24	0.08
ATTO 680	796	568	681	698	125000	0.3	0.17
ATTO 700	836	608	700	716	120000	0.26	0.41
ATTO MB2	626	400	668		100000	0.08	0.24

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); 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 $M^{-1}cm^{-1}$; CF260 = ε 260/ ε max; CF280 = ε 280/ ε max;