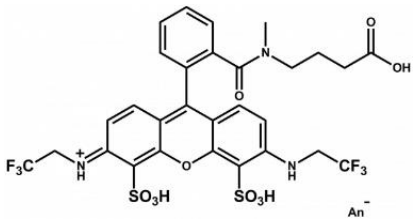
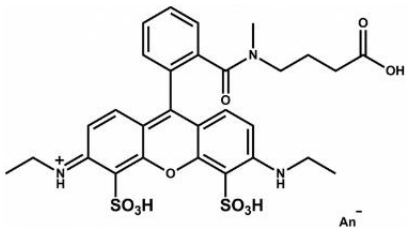
ATTO 514

ATTO 514 is a patented fluorescent marker from the rhodamine dye family with excellent water solubility.

A structural particularity of ATTO 514 is a trifluoroethyl substituent on each amino group of the rhodamine chromophore. These strongly electron-withdrawing groups affect both optical and chemical properties of the dye.

Absorption and fluorescence shift about 20 nm to shorter wavelengths. This is shown by a comparison of the spectroscopic data of ATTO 514 and ATTO 532 - measured at 22 °C in aqueous solution (PBS, pH 7.4):

	ATTO 514 carboxy	ATTO 532 carboxy
structure		
λ_{abs}	511 nm	532 nm
λ_{fl}	532 nm	552 nm

The trifluoroethyl substitution also affects the chemical stability of the o-carboxamide group.

When using e.g. ATTO 514 NHS-ester for labeling amino groups in biomolecules, occasionally a cleavage of the chromophore from the conjugate was observed when concentrating the purified coupling product in triethylammonium acetate buffer (TEAA buffer). That is, only the aminobutyric acid residue remains bound to the biomolecule and causes an increase in mass of $\Delta m \approx 100$ daltons over the unlabeled protein or oligonucleotide.

For reactions with ATTO 514 derivatives, please refer to our ATTO-TEC coupling procedures, e.g. in the case of NHS ester, the short reaction time of 30 - 60 minutes at pH 8.3.

We make the following recommendations for the workup of the ATTO 514 biomolecule conjugate formed:

1) Use PBS buffer instead of TEAA buffer.
2) Reduce the molarity of the buffer, e.g. 0.1 M instead of 1 M.
3) Adjust the pH to 6 - 7 before concentrating.
4) Use gentle parameters to concentrate the conjugate solution, such as 25 °C at a vacuum below 10 mbar.