ATTO-TEC

Revised: July 29, 2019

Product Information: ATTO Tetrazines
(for bioorthogonal TCO-tetrazine ligation)

Compound	Storage information	Shelf Life
Tetrazine modified reactive label, lyophilized or crystalline solid	Freeze upon receipt < -20°C Protect from light and moisture	When stored as indicated, ATTO tetrazines are stable for at least 3 years.
For optical properties see Table 1 on page 2.		

Introduction:

Tetrazines readily react with strained or vinyl alkenes in a highly selective and bioorthogonal way. All **ATTO tetrazines** are based on **6-methyl-3-aryl tetrazine** (**MeTet**) which provides high stability in aqueous media and still shows very high reaction rates. The ligation of **ATTO tetrazines** with e.g. trans-cyclooctenes (TCO) proceeds with rate constants of up to 1000 M^{-1} s^{-1}, about three orders of magnitude faster than typical azide alkyne cycloaddition (AAC) or strain promoted azide alkyne cycloaddition (SPAAC). This high reactivity is a prerequisite for any application performed under highly diluted conditions such as protein conjugations.
The TCO-tetrazine ligation can be considered as a strain promoted inverse electron demand Diels-Alder cycloaddition (SPIEDAC), forming a dihydropyridazine derivative[1] (Figure 1).

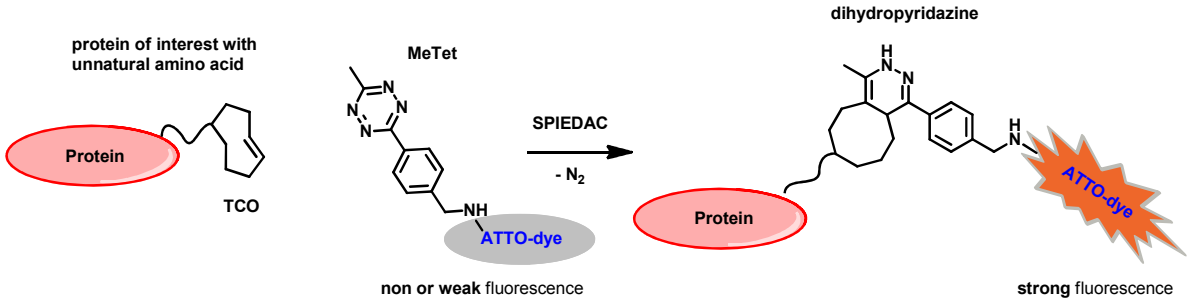

Figure 1: TCO-tetrazine ligation with ATTO-dye labeled tetrazine.

For many ATTO-dyes the fluorescence is heavily quenched by the tetrazine residue. After the conjugation reaction has taken place the initially strongly reduced emission is restored, making them fluorogenic probes[2]. This is reflected by turn on ratios of up to a factor 30 depending on the dye and reactant.

ATTO tetrazines are available for a variety of fluorophores. They are provided in units of 0.2 and 0.5 mg (Table 1).

Protocol for TCO-tetrazine ligation:

The following protocol describes the labeling procedure for **ATTO tetrazines** with TCO labeled biomolecules, e.g. proteins.

Required Materials

- **Component A**: Dissolve the **ATTO tetrazine** in the appropriate amount of DMSO to obtain a 0.5 - 1 mM solution. Aliquots of this solution may be stored at -20 °C.
  Note: The shelf life of such solutions will be significantly reduced depending on the quality of the solvent used.
- **Component B:** The TCO-carrying protein should be dissolved in a buffer like PBS, HBSS (Hank's balanced salt solution) or in case of transfected cells in an appropriate cell growth medium, e.g. DMEM (Dulbecco's modified Eagle medium).

Tetrazine conjugation

- Pipette the appropriate amount of **Component A** to **Component B** to achieve a final tetrazine concentration of 1 – 3 µM.
- Incubate for 10 – 30 min at 4 °C, 25 °C or 37 °C depending on the application.
- After successful ligation, excess amount of **ATTO tetrazine** can be washed away using PBS, HBSS or DMEM (2-3 times). In the case of fluorogenic tetrazines, like ATTO 425 MeTet, ATTO 488 MeTet, ATTO 532 MeTet and ATTO 655 MeTet, the washing step may be omitted.
- Cell fixation can be carried out with 4 % formaldehyde in PBS for 15 min followed by three wash cycles with HBSS.
- No fixation for live cell staining.

Table 1: ATTO-dye labeled tetrazines:

Dye	Order #		MW	M^+	λ_{abs}	λ_{em}	ε_{max}
	0.2 mg	0.5 mg					
ATTO 425	AD 425-2502	AD 425-2505	585	586	439	485	45000
ATTO 488	AD 488-2502	AD 488-2505	887	773	500	520	90000
ATTO 532	AD 532-2502	AD 532-2505	943	829	532	552	115000
ATTO 550	AD 550-2502	AD 550-2505	891	777	554	567	120000
ATTO 647**N**	AD 647**N**-2502	AD 647**N**-2505	930	830	646	664	150000
ATTO 655	AD 655-2502	AD 655-2505	825	711	663	680	125000

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}.

Literature:

[1] M. Blackman, M. Royzen, J. Fox, *Tetrazine ligation: fast bioconjugation based on inverse-electron-demand Diels-Alder reactivity*, Journal of the American Chemical Society **130**, 13518 (2008).
N. Devaraj, R. Weissleder, *Biomedical applications of tetrazine cycloadditions*, Accounts of Chemical Research **44**, 816 (2011).
T. Plass, S. Milles, C. Koehler, J. Szymanski, R. Mueller, M. Wiessler, C. Schultz, E. Lemke, *Amino acids for Diels-Alder reactions in living cells*, Angewandte Chemie International Edition **51**, 4166 (2012).

[2] G. Beliu, A. Kurz, A. Kuhlemann, L. Behringer-Pliess, M. Meub, N. Wolf, J. Seibel, Z.-D. Shi, M. Schnermann, J. Grimm, L. Lavis, S. Doose, M. Sauer, *Bioorthogonal labeling with tetrazine-dyes for super-resolution microscopy*, Communications Biology **2**, 261 (2019).

Detailed information on each individual dye including risk and safety data as well as certificate of analysis (CoA) can be downloaded from our website at www.atto-tec.com.

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