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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Storage information</th>
<th>Shelf Life</th>
</tr>
</thead>
</table>
| 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.](image)

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 490LS 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:**

<table>
<thead>
<tr>
<th>Dye</th>
<th>Order #</th>
<th>0.2 mg</th>
<th>0.5 mg</th>
<th>MW</th>
<th>M⁺</th>
<th>λ abs</th>
<th>λ em</th>
<th>ε max</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATTO 425</td>
<td>AD 425-2502</td>
<td>AD 425-2505</td>
<td>585</td>
<td>586</td>
<td>439</td>
<td>485</td>
<td>45000</td>
<td></td>
</tr>
<tr>
<td>ATTO 488</td>
<td>AD 488-2502</td>
<td>AD 488-2505</td>
<td>887</td>
<td>773</td>
<td>500</td>
<td>520</td>
<td>90000</td>
<td></td>
</tr>
<tr>
<td>ATTO 490LS</td>
<td>AD 490LS-2502</td>
<td>AD 490LS-2505</td>
<td>879</td>
<td>857</td>
<td>495</td>
<td>658</td>
<td>40000</td>
<td></td>
</tr>
<tr>
<td>ATTO 532</td>
<td>AD 532-2502</td>
<td>AD 532-2505</td>
<td>943</td>
<td>829</td>
<td>532</td>
<td>552</td>
<td>115000</td>
<td></td>
</tr>
<tr>
<td>ATTO 550</td>
<td>AD 550-2502</td>
<td>AD 550-2505</td>
<td>891</td>
<td>777</td>
<td>554</td>
<td>567</td>
<td>120000</td>
<td></td>
</tr>
<tr>
<td>ATTO 647N</td>
<td>AD 647N-2502</td>
<td>AD 647N-2505</td>
<td>930</td>
<td>830</td>
<td>646</td>
<td>664</td>
<td>150000</td>
<td></td>
</tr>
<tr>
<td>ATTO 655</td>
<td>AD 655-2502</td>
<td>AD 655-2505</td>
<td>825</td>
<td>711</td>
<td>663</td>
<td>680</td>
<td>125000</td>
<td></td>
</tr>
</tbody>
</table>

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 max: 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:**


**Contact Information:**

**ATTO-TEC GmbH**
Am Eichenhang 50
57076 Siegen
Germany

ATTO-TEC products are high-quality reagents intended for research purposes only. These compounds must be used by, or under the direct supervision of, technically qualified individuals experienced in handling potentially hazardous chemicals. We refer to the Material Safety Data Sheet (MSDS) provided with each product.

Phone: +49(0)271-23853-0
Fax: +49(0)271-23853-11
e-mail: info@atto-tec.com
http: www.atto-tec.com

Additional information on ATTO-TEC and its entire product range is available on our website [www.atto-tec.com](http://www.atto-tec.com). For further questions contact us directly by e-mail or fax.