SHNH (HyNic for Technetium Labeling)

$C_{10}H_{11}ClN_4O_4$; Mol. Wt.: 286.67

Cat. No.: S-1001-010

Storage: Desiccated: -15° to -25°C

**Introduction**

SHNH is a bifunctional aromatic hydrazine linker used to incorporate HyNic (6-hydrazinonicotinamide) linkers onto biomolecules through amino groups via an activated ester (i.e., NHS ester, Figure 1).

![Figure 1: Scheme presenting the modification of a protein with SHNH.](image)

The number of HyNic linkers incorporated on biomolecules can be quantified colorimetrically by reaction with 2-sulfobenzaldehyde. The product yields a chromophore that absorbs at 350 nm with a molar extinction coefficient of 28,500 L/(mol*cm).

**Additional materials required**

- **Reagents**
  - Zeba™ Desalting Columns
  - Modification Buffer (10X)
  - Anhydrous DMF

- **Equipment**
  - Variable-speed bench-top centrifuge
  - Spectrophotometer or Plate Reader
  - 1.5 mL microcentrifuge tubes

**Modification Procedure**

**A. Desalting**

1. Desalt/buffer exchange the protein into 1X Modification Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 8.0). If needed, refer to the Protein Desalting Protocol.

**Notes:**

   a) It is necessary to remove all free amine-containing contaminants, e.g. tris or glycine, from the protein solution before modification.

   b) High-level buffering capacity, i.e. 100 mM phosphate, is necessary for successful modification.

   c) For desalting proteins, Zeba Desalting Columns are recommended.

**B. Determine the concentration of the desalted protein**

1. Determine the concentration of the protein to be modified using a spectrophotometer and the known E1% (280 nm). Alternatively, a Bradford assay or BCA assay can be used if the protein extinction coefficient is not known.

2. Adjust the concentration to 1.0 – 4.0 mg/mL in 1X Modification Buffer, pH 8.0, if necessary.

**C. Prepare SHNH Solution**

1. Prepare a stock solution of SHNH in anhydrous DMF (or DMSO) by dissolving 2 – 4 mg of SHNH in 100 µL anhydrous DMF.

   **Note:** The SHNH/DMF stock solution must be used immediately.

<table>
<thead>
<tr>
<th>IgG Concentration (mg/mL)</th>
<th>SHNH Mole Equivalents Added</th>
<th>Determined Ratio of HyNic/Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>20</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>8.2</td>
</tr>
<tr>
<td>4.0</td>
<td>15</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>7.8</td>
</tr>
</tbody>
</table>

   **Table 1:** Recommended equivalents of SHNH linker to add to proteins at increasing concentrations to incorporate a specific linker molar substitution ratio (MSR). An MSR of 4 – 7 is recommended for antibodies.

**D. Modification of a protein**

1. Using Table 1 as a guide, or the with the aid of the Protein Modification Calculator, add the required volume of SHNH/DMF to the protein solution.

   **Notes:** Depending on the size of the protein and the desired level of modification, the number of equivalents should be adjusted.

2. Allow the reaction to incubate at room temperature for 1.5 – 2.0 hours.

**E. Desalting procedure**

1. Desalt/buffer exchange the protein into Conjugation Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 6.0) if it will be conjugated to a 4FB-modified biomolecule. Otherwise, desalt the modified protein into a buffer compatible with the intended application. If needed, refer to the Protein Desalting Protocol.

   **Note:** The protein concentration cannot be determined spectrophotometrically after modification with SHNH due to the 280 nm absorbance of the aromatic linkers. A BCA or Bradford protein assay is recommended to determine concentration after labeling.
F. Quantifying the molar substitution ratio (MSR)

1. The molar substitution ratio (MSR) can be determined using a colorimetric reaction with 2-sulfobenzaldehyde, as outlined in Figure 2 below. Addition of a HyNic-modified biomolecule to 2-sulfobenzaldehyde yields an aromatic hydrazone that absorbs at 350 nm. Refer to the HyNic-Protein MSR Calculator as well as the protocol that is appropriate for your lab equipment: HyNic Protein MSR Instructions.

![Colorimetric reaction](image)

Figure 2: Colorimetric reaction used to quantify number of HyNic linkers on a biomolecule.

2. The biomolecule is now modified with HyNic and ready for labeling with Technetium-99M or conjugation to 4FB-modified biomolecules or surfaces.

Note: HyNic-modified proteins should be labeled with Technetium-99M or conjugated to a 4FB-modified molecule or surface immediately following desalting and protein concentration determination.

Application Notes

- Performing a Bradford assay
- Performing a BCA protein assay
- Troubleshooting Guide