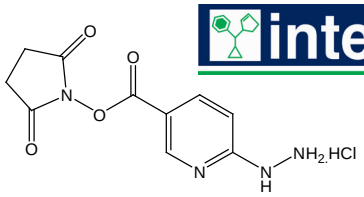
SHNH

$C_{10}H_{11}ClN_4O_4$ MW 286.67
S-1001-010
S-9001-1 Kit



Instructions

Introduction:

SHNH (S-1001-010) is an bifunctional aromatic hydrazine linker used to incorporate HyNic (6-hydrazinonicotinamide) moieties onto biomolecules through their amino group via an activated ester (i.e. NHS; Figure 1). HyNic groups were developed to link Tc-99M to proteins for *in vivo* imaging.[1-4]

Figure 1: Scheme presenting the modification of a protein with SHNH.

The number of HyNic moieties incorporated on biomolecules can be quantified calorimetrically on reaction with 2-sulfobenzaldehyde (Solulink catalog# S-2005-100). The product yields a chromophore that absorbs at A350 with a molar extinction coefficient of 18000 (Figure 2). Procedures and calculators to guide users through this process can be found at **http://www.SoluLinK.com/protocols.php**

Reagents	Equipment
Desalting Spin columns (cat # S-4004-025)	Variable-speed bench-top microcentrifuge
Modification Buffer (cat # S-4003-005)	Spectrophotometer or Plate Reader
DMF (anhydrous) (cat # S-4001-005)	1.5 mL microcentrifuge tubes

Note: This Protocol and all links below can be downloaded at **http://www.SoluLinK.com/protocols.php**

Modification Procedure

Desalting procedure (More detailed protocols at LINK)

1. Desalt/buffer exchange the protein into Modification Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 7.4).

Notes:

a) It is necessary to remove all free amine-containing contaminants, *e.g.* tris, 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 SoluLink recommends Pierce Zeba Desalt Spin columns (# 89882) or Sartorius Vivaspin diafiltration units (#VS0112). Refer to desalting protocol for either apparatus.

A. Determine the concentration of the protein (More detailed protocols at LINK)

1. Determine the concentration of the protein to be modified using the BCA assay (ThermoScientific, #23223) or spectrophotometrically if the extinction coefficient of the protein is known.
2. Bring the concentration to 1-4 mg/mL in Modification Buffer pH 7.4

B. Prepare the SHNH Solution

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

Note:

a) The SHNH/DMF stock solution must be used immediately.

C. Modification of of protein (More detailed protocols at LINK) and of oligonucleotides (LINK)

1. Using Table 1 as a guide, add the requisite volume of SHNH/DMF to the protein solution.

Notes:

a) Depending on the size of the protein and the desired level of modification, the number of equivalents should be adjusted.

2. Allow reaction to incubate at room temperature for 1.5-2 hours.

IgG concentration	SHNH mole equivalents added	Determined ratio of HyNic/protein
1.0	20	5.5
	30	8.2
4.0	15	4.7
	20	6.4
	25	7.8

Table 1: Recommended equivalents of SHNH linker to add to proteins at increasing concentrations to incorporate a specific linker substitution ratio.

D. Desalting procedure (detailed protocols at LINK)

1. Desalt/buffer exchange the protein into Conjugation Buffer (0.1M sodium phosphate, 0.15M sodium chloride, pH 6.0). For proteins SoluLinK recommends Pierce Zeba Desalt Spin columns (# 89882) or for oligonucleotides SoluLinK recommends Sartorius Vivaspin diafiltration units (#VS0112). Refer to desalting protocol for either apparatus.

E. Quantifying modification level (detailed protocols at LINK)

1. The molar substitution ratio (MSR) can be determined using a colorimetric reaction outlined in Figure 2. Addition of 2-sulfobenzaldehyde to a HyNic-modified biomolecule yields a bis-arylhydrazone that absorbs at 350 nm. Calculator/protocols can be downloaded from: LINK

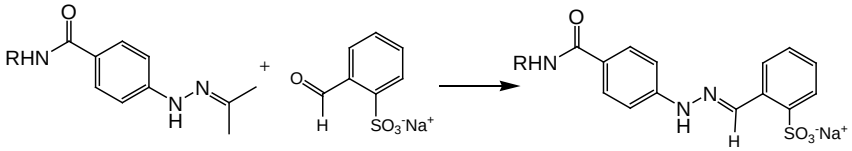


Figure 2: Colorimetric reaction used to quantify number of HyNic linkers on a biomolecule

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

Note:

a) HyNic-modified oligonucleotides are not stable and must be conjugated or immobilized immediately following desalting.

Troubleshooting

Problem	Possible Cause	Solution
Protein was not modified or poorly modified.	Protein has been contaminated with amine containing compounds The concentration of the protein was too low	Desalt the protein more thoroughly with a new Zeba Spin column Increase the concentration of the protein to >2.0 mg/mL
SHNH was hydrolyzed	Wet or poor quality DMF/DMSO hydrolyzed the NHS ester	Use a good quality anhydrous DMF/DMSO to solubilize the SHNH molecule.

Stability

It is recommended to use the HyNic modified protein immediately. If long term storage is required it is recommended to store the modified protein <-20 ^{O}C and perform a time course stability study.

Related SoluLink Products

S-9002-1	S-HyNic Kit	S-4004-025	VivaSpin diafiltration device	S-2005-100	2-sulfo-benzaldehyde
S-1002-010	S-HyNic	S-4001-005	DMF anhydrous	S-4023-005	Aniline
S-4003-005	Modification Buffer	S-4002-005	Conjugation Buffer	S-4024-005	Aniline conjugation buffer

References

1. Preparation and Preliminary Evaluation of 99mTc Labeled Fragment E1 for Thrombus Imaging, L.C. Knight, M.J. Abrams, D.A. Schwartz, M.M. Hauser, M. Kollman, F.E. Gaul, D.A. Rauh and A.H. Maurer, J. Nucl. Med. 33, 710 (1991).
2. Preparation of Hydrazino-Modified Proteins and Their Use for the Synthesis of 99mTc Protein Conjugates, D.A. Schwartz, M.J. Abrams, M.M. Hauser, F.E. Gaul, D. Rauh, J.A. Zubieta and S.K. Larsen, Bioconjugate Chem. 2, 333 (1991).
3. 11) 99mTc Human Polyclonal IgG Radiolabeled via the Hydrazino Nicotinamide Derivative for Imaging Focal Sites of Infection in Rats. M.J. Abrams, M. Juweid, C.I. tenKate, D. Schwartz, M.J. Hauser, F.E. Gaul, A.J. Fuccello, R.H. Rubin, H.W. Strauss, and A.J. Fischman, J. Nucl. Med. 31, 2022 (1990).
4. For an extensive list of references see PubMed or Google Scholar: keyword HYNIC