

Protein / Peptide Conjugation Protocol



Materials Required

Reagents	Catalog #	Equipment
Zeba Desalting Columns	S-4024	Variable-speed bench-top microcentrifuge
Modification Buffer	S-4003	Spectrophotometer or Nanodrop
Conjugation Buffer	S-4002	1.5 mL microcentrifuge tubes
DMF (anhydrous)*	S-4001	
S-4FB or Sulfo-S-4FB	S-1006 or S-1008	
2-Sulfobenzaldehyde	S-2005	

*DMF is required only if using S-4FB

In addition to the above materials, user must obtain desired protein and HyNic-peptide before proceeding. Synthesizing peptides with N-terminus HyNic requires the utilization of BOC-HNA (Catalog # S-3003) or C-terminus peptides requires FMOC-Lysine- ϵ [BOC-HNA]-OH (Catalog # S-3034). HyNic-peptides may also be ordered directly through Solulink. A primer describing examples, use and methods of preparation of HyNic peptides can be downloaded here: http://www.solulink.com/white_papers/peptide.pdf.

Protein Modification Procedure

A. Desalting procedure

1. Desalt/buffer exchange the protein into Modification Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 7.4); if needed, refer to the [Protein Desalting Protocol](#).
 - 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 and buffer exchanging proteins, Solulink recommends Zeba Desalting Columns (#S-4024). Refer to desalting protocol for apparatus.

B. Determine the concentration of the protein

1. Determine the concentration of the protein to be modified using the [BCA assay](#) or [Bradford assay](#). Alternatively, if the extinction coefficient of the protein is known then the concentration can be determined spectrophotometrically using the A_{280} .
2. Bring the concentration to 1.5-5 mg/mL in Modification Buffer, pH 7.4.
 - a) If the solution is too concentrated, add Modification Buffer, pH 7.4 until desired concentration is achieved.
 - b) If the solution is too dilute, a Sartorius Vivaspin dialfiltration unit (S-4004) can be used to concentrate the protein.

C. Prepare a S-4FB/DMF stock solution

1. Prepare a stock solution of S-4FB in anhydrous DMF (or DMSO) by dissolving 2-4 mg of S-4FB in 100 μ L anhydrous DMF. Alternatively, Sulfo-S-4FB may be used instead and dissolved into an aqueous buffer (i.e., Modification Buffer, pH 7.4).
 - a) The S-4FB/DMF stock solution is stable for 2 weeks if prepared with anhydrous DMF (Solulink catalog# S-4001) and stored desiccated.
 - b) The Sulfo-S-4FB/aqueous buffer solution is not stable and must be used within 30 minutes and discarded.

D. Modification of the Protein

1. Add the requisite volume of S-4FB/DMF or Sulfo-S-4FB/aqueous buffer stock to the protein solution. The recommended amount of equivalents of S-4FB or Sulfo-S-4FB to protein is molecular weight and concentration dependent.
 - a) **Table 1** shows an example of 4FB incorporation against varying equivalents and concentrations on an antibody.

- b) Lower molecular weight proteins need fewer equivalents of S-4FB / Sulfo-S-4FB to achieve the same 4FB/Protein ratio.
- Refer to the “Modification” worksheet of the [Protein / Peptide Conjugation Calculator](#), if needed.
 - Be sure to use the correct values for S-4FB or Sulfo-S-4FB in the **Reagent Information** section of the calculator.
 - Allow reaction to incubate at room temperature for 2.0 hours.

IgG concentration (mg/mL)	S-4FB molar equivalents added	Determined ratio of 4FB/protein
1.0	5	2.38
	10	4.73
	15	6.20
2.5	5	3.08
	10	6.58
5.0	5	3.74
	10	6.80

Table 1: The number of 4FB groups incorporated on an antibody is dependent on the number of mole equivalents S-4FB added and the protein concentration. This table can be used as a general guide for modification of any protein with a succinimidyl-based modification reagent.

E. Desalting procedure

- Desalt/buffer exchange the protein into Modification Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 7.4); if needed, refer to the [Protein Desalting Protocol](#).

F. Quantifying modification level

- The molar substitution ratio (MSR) can be determined using a colorimetric reaction outlined in Figure 2. Addition of 2-hydrazinopyridine to a 4FB-modified biomolecules yields a compound that absorbs at 350 nm.
- Refer to the “Modification” worksheet on the [Protein / Peptide Conjugation Calculator](#) well as the protocol that is appropriate for your lab equipment: [4FB Colorimetric Protein MSR Assay with a Nanodrop Protocol](#), or [4FB Colorimetric Protein MSR Assay with a Spectrophotometer Protocol](#), depending on your application.

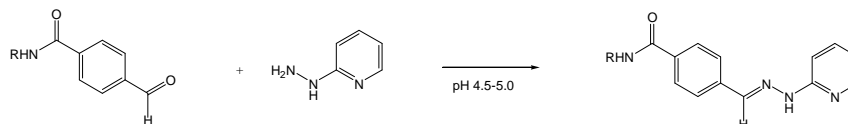


Figure 2: Colorimetric reaction used to quantify number of 4FB moieties on a biomolecule

- The biomolecule is now 4FB-modified and ready for conjugation to HyNic-modified biomolecules and surfaces.

Protein / Peptide Conjugation Protocol

A. HyNic-peptide preparation

- HyNic-peptide should be dissolved into a 10-20 mg/mL aqueous solution.
 - Depending on the peptide’s solubility, it may be directly dissolved using 1x Conjugation Buffer, pH 6.0.
 - If it is not soluble in aqueous solutions, it may be first dissolved using DMF and then an equal volume of 1x Conjugation Buffer, pH 6.0 should be added.
 - The final peptide solution MUST be soluble in an aqueous buffer or partly aqueous buffer before conjugation.

B. Conjugation of 4FB-protein and HyNic-peptide

- Refer to the “Conjugation” worksheet of the [Protein-Peptide Conjugation Calculator](#) to determine volume of each reagent solution to add to combine.
- Add indicated amount of TurboLink Catalyst Buffer.
- Allow reaction to incubate at room temperature for 2.0 hours.

C. Peptide purification and buffer exchange

- The Protein-Peptide conjugate can be purified from excess peptide by using a Diafiltration spin column (Cat#: S-4004) with a molecular weight cut off that is larger than the peptide. Additionally, the conjugate may be buffered exchanged into a buffer of choice using this apparatus. Protocol for using the diafiltration unit can be found in the [Desalting Protocol](#).