Oligonucleotide / Peptide Conjugation Protocol

Materials Required

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Catalog #</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diafiltration spin columns</td>
<td>S-4004</td>
<td>Variable-speed bench-top microcentrifuge</td>
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<tr>
<td>Modification Buffer</td>
<td>S-4003</td>
<td>Spectrophotometer or Nanodrop</td>
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<tr>
<td>Conjugation Buffer</td>
<td>S-4002</td>
<td>1.5 mL microcentrifuge tubes</td>
</tr>
<tr>
<td>DMF (anhydrous)*</td>
<td>S-4001</td>
<td></td>
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<tr>
<td>S-4FB or Sulfo-S-4FB</td>
<td>S-1006 or S-1008</td>
<td></td>
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<tr>
<td>2-Sulfobenzaldehyde</td>
<td>S-2005</td>
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*DMF is required only if using S-4FB

In addition to the above materials, user must obtain amino-oligonucleotide 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](http://www.solulink.com/white_papers/peptide.pdf).

Amino-oligonucleotide Modification Procedure

A. Desalting procedure

1. Desalt / buffer exchange the oligonucleotide into Modification Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 8.0); if needed, refer to the Oligonucleotide Desalting Protocol.

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 oligonucleotides, Solulink recommends Sartorius Vivaspin diafiltration units (#VS0112). Refer to desalting protocol for apparatus.

B. Determine the concentration of the oligonucleotide

1. Fill two microcentrifuge tubes with 998 µL of H2O. Create a “Blank” tube by adding 2 µL of chosen buffer into the first tube. Add 2 µL of the oligonucleotide solution to the second tube.
2. At 260 nm, blank the spectrophotometer using the “Blank” tube, then take the reading of the second tube.
3. Divide that number in half to obtain the concentration of the oligonucleotide solution (OD260/µL).
4. Adjust the concentration to be between 0.250 and 0.500 OD260/µL in Modification Buffer, pH 8.0.

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 µL anhydrous DMF. Alternatively, Sulfo-S-4FB may be used instead and dissolved into an aqueous buffer (i.e., Modification Buffer, pH 8.0).

Note:
   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 oligonucleotide

1. Add the requisite volume of S-4FB/DMF or Sulfo-S-4FB/aqueous buffer stock to the oligonucleotide solution. The recommended amount of equivalents of S-4FB or Sulfo-S-4FB to oligonucleotide is 20x and may be adjusted according to project. Refer to the “Modification” worksheet of the 4FB-Oligonucleotide / HyNic-Peptide Conjugation Calculator, if needed.
Note:
   a) Be sure to use the correct values for S-4FB or Sulfo-S-4FB in the **Reagent Information** section of the calculator
2. Allow reaction to incubate at room temperature for 2.0 hours.

E. **Desalting procedure**
   1. Dilute reaction mixture to 500 µL with Conjugation Buffer.
   2. Desalt/buffer exchange the oligonucleotide into Conjugation Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 6.0); if needed, refer to the **Oligonucleotide Desalting Protocol**.

F. **Quantifying modification level**
   1. The molar substitution ratio (MSR) can be determined using a colorimetric reaction outlined in Figure 2. Addition of 2-hydraizinopyridine to a 4FB-modified biomolecules yields a compound that absorbs at 350 nm. Refer to the “Modification” worksheet on the **Oligonucleotide-Peptide Conjugation Calculator** as well as the protocol that is appropriate for your lab equipment: **2-HP Oligonucleotide MSR with a Nanodrop Protocol**, or **2-HP Oligonucleotide MSR with a Spectrophotometer Protocol**, depending on your application.

   ![Figure 2](#)

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

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

**Oligonucleotide / Peptide Conjugation Protocol**

A. **HyNic-peptide preparation**
   1. HyNic-peptide should be dissolved into a 10-20 mg/mL aqueous solution.
      a. Depending on the peptide's solubility, it may be directly dissolved using 1x Conjugation Buffer, pH 6.0.
      b. 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.
      c. The final peptide solution MUST be soluble in an aqueous buffer or partly aqueous buffer before conjugation.

B. **Conjugation of 4FB-oligonucleotide and HyNic-peptide**
   1. Refer to the “Conjugation” worksheet of the **Oligonucleotide-Peptide Conjugation Calculator** to determine volume of each reagent solution to add to combine.
   2. Add indicated amount of TurboLink Catalyst Buffer.
   3. Allow reaction to incubate at room temperature for 2.0 hours.

C. **Peptide purification and buffer exchange**
   1. The Oligonucleotide-Peptide conjugate can be purified from excess peptide by using a Sartorius Vivaspin dialfiltration units with a molecular weight cut off that is larger than the peptide. Additionally, the conjugate may be buffer exchanged into a buffer of choice using this apparatus. Protocol for using the Sartorius Vivaspin dialfiltration unit can be found in the **Oligonucleotide Desalting Protocol**.