



Protein-Oligonucleotide Conjugation Kit

Technical Manual

Catalog # S-9011-1

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Safety Information

WARNING – CHEMICAL HAZARD. Some chemicals used can be potentially hazardous, and can cause injury or illness.

- Read and understand the Material Safety Data Sheets (MSDS) available at Solulink.com before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (e.g. safety glasses, gloves, or clothing). For additional safety guidelines consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s clean-up procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling and disposal.

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I. Introduction

a. Product Description

The Protein-Oligo Conjugation Kit is designed to conjugate two separate protein-oligo conjugates. It includes all of the necessary components and protocols for easy and specific crosslinking of any protein with any oligo up to 100 base pairs in length. This kit is a flexible so that researchers with little or no conjugation experience can make their own custom protein-oligo conjugate to suit their needs.

The SoluLink bioconjugation method guarantees >95% conversion of protein to conjugate when more than 4 molar equivalents of oligo are added. High conversion rates, coupled with the unique UV traceable bond formed during crosslinking, allows for easy purification and identification of the conjugate from the excess oligo using size exclusion purification methods such as FPLC or diafiltration.

Each kit contains the material to synthesize two conjugation reactions for any immunoPCR or ligation-type assay in just under 4 hours each; each reaction yields between 40-60% conjugate after purification.

b. The SoluLink Bioconjugation Method

The Protein-Oligo Conjugation Kit uses SoluLink's superior Bioconjugation Method to prepare protein-oligonucleotide conjugates in 3 easy-to-perform steps (Figure 1). The first step is the modification of the protein with our HyNic crosslinker, followed by the formation of the 4FB modified oligonucleotide. Finally, simple mixing of the two modified biomolecules will result in the formation of a stable, UV-traceable bond formed by the reaction of a HyNic modified protein with a 4FB modified oligonucleotide.

This technology has many practical advantages compared to previous crosslinking methods:

1)The reaction is high yielding. Routinely yields of conjugate are 40-60% based on starting protein.

2)The reaction is efficient: Only 1.5-2 molar equivalents of oligo is necessary for each oligo desired on the protein. When conjugating more than two oligos per protein, >95% of the protein is conjugated.

3)The conjugate bond is extremely stable: The conjugate bond is stable to 92°C and pH 2.0-10.0.

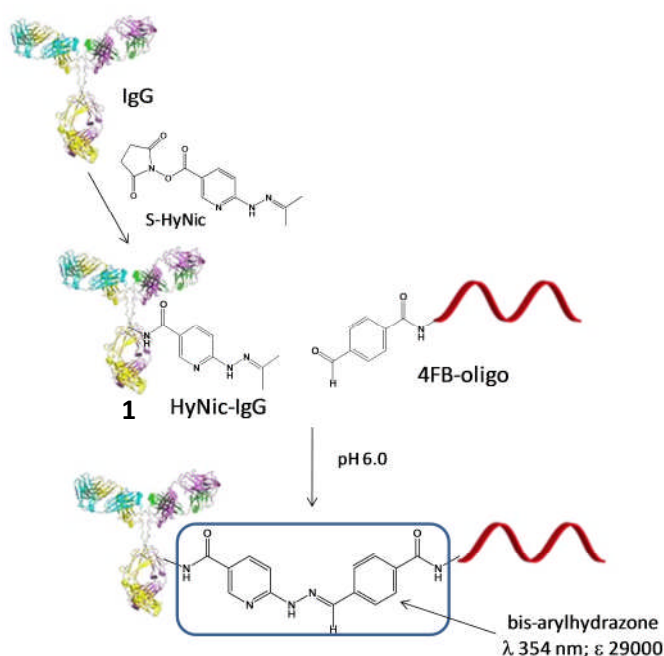


Figure 1: Schematic representation of the two step process to prepare an antibody-oligonucleotide conjugate using SoluLink's Bioconjugation chemistry. Initially an antibody is modified with S-HyNic to incorporate HyNic groups and subsequently the HyNic-modified antibody is reacted with a 4FB-modified oligonucleotide.

4)The reaction conditions are mild and do not cause any protein denaturation: Unlike thiol-based conjugation protocols, where reducing reagents are required that can compromise the activity of proteins by cleaving disulfide bonds, the HyNic-4FB conjugation couple leaves disulfide bonds intact. No metals, oxidation or reducing reagents are required.

5)The conjugation is traceable spectrophotometrically. The HyNic-4FB conjugate bond is UV traceable- it absorbs at 354 nm and has a molar extinction coefficient of 29000. This allows for real time spectrophotometric monitoring of the conjugation reaction, the ability to ‘visualize’ the conjugate during purification using a UV or photodiode array detector, and quantification of conjugation.

6)The modifications of both the HyNic linker on the protein and the 4FB linker on the oligonucleotide are quantifiable using colorimetric assays. The reproducibility of any reaction is facilitated by accurate characterization of all components. The Molar Substitution Ratio (MSR) of linker groups, *i.e.* the number of HyNic linkers per protein, can be quantified colorimetrically. This kit contains all the reagents necessary to determine the MSRs for both the protein and the oligo.

II. Protein-Oligonucleotide Conjugates: A Review

The diversity and specificity of antibodies combined with the specificity of hybridization of oligonucleotides results in unlimited numbers of specific protein detection reagents whose applications are addressed below.

The use of oligo-protein conjugates was initially demonstrated by Sano *et al.*¹ for protein detection by a technique called immuno-PCR (Polymer Chain Reaction) where a 100mer oligo/antibody conjugate was allowed to bind to its ligand and amplified by PCR demonstrating extremely sensitive protein detection. Since this initial publication there has been a need for a straightforward, efficient and high yielding method for the preparation of these conjugates.

The first generation immuno-PCR protocol was plagued by high background due to non-specific binding of the conjugate and the extreme sensitivity of PCR. This has been overcome by the Proximal Ligation Assay (PLA) developed by Fredriksson and Lundegren.² In the PLA assay, two antibodies to different epitopes are conjugated to a 40mer 5'-phosphorylated oligonucleotide through the 3'-end and 60mer oligonucleotide conjugated through its 5'-terminus. The two oligo/antibody conjugates are incubated with the sample, allowed to bind to their respective epitopes, the mixture is washed and then incubated with a 'splint' oligo that hybridizes across the two oligonucleotides that is subsequently ligated. Following ligation, PCR is performed on the ligated oligo generating a quantifiable signal. In subsequent work the oligo/antibody conjugates used by Fredriksson *et al.* and others used conjugates prepared by SoluLink using the HyNic-4FB Conjugation Method.³⁻⁶ Kozlov *et al.*⁷ also describe the use of oligonucleotide/antibody conjugates for the sensitive detection of proteins.

Additionally, antibody/oligonucleotide conjugates have been used for capture of antigens and subsequent addressing to antibody arrays for multiplex detection of proteins as well for cell sorting on the same diagnostic platform.^{8,9} Oligonucleotide/protein conjugates have been also been used in vaccines to increase adjuvanticity using CpG oligonucleotide/protein conjugates.¹⁰⁻¹²

III. Accessing 4FB-modified Oligonucleotides

Stable and disulfide-cleavable 4FB oligonucleotides can be obtained in several ways:

1. 5'-4FB oligonucleotide

- 4FB-phosphoramidite:** 4FB-Phosphoramidite (**1**; Figure 2) is available for incorporation of 5'-4FB groups during oligonucleotide solid phase synthesis. Standard coupling protocols are used and the yields are similar to any amino modifier. The 4FB Phosphoramidite may be purchased directly (SoluLink catalog #S-1005) or you may order 5'-4FB oligonucleotides directly from SoluLink.
- 5'-amino oligonucleotides:** 5'-amino oligonucleotides may be converted to 5'-4FB modified oligonucleotides in a straightforward high yielding modification step with S-4FB (**2**; Figure 2).

The Protein-Oligo Conjugation Kit includes S-4FB and all the reagents and materials required to convert a 5'-amino oligonucleotide to a 5'-4FB-oligonucleotide.

2. 3'-4FB oligonucleotide:

3'-Amino oligonucleotides are converted to 3'-4FB modified oligonucleotides in an easy, high yielding modification step with S-4FB (**2**; Figure 2).

The Protein-Oligo Conjugation Kit includes S-4FB and all the reagents and disposables required to convert a 3'-amino oligonucleotide to a 3'-4FB-oligonucleotide.

- 5'- and 3'-4FB disulfide-cleavable oligonucleotides:** 5'- and 3'-amino oligonucleotides may be converted to disulfide-cleavable oligonucleotides using S-SS-4FB (**3**; Figure 2) in an easy, high yielding modification step. This product is available separately (SoluLink catalog #S-1037).

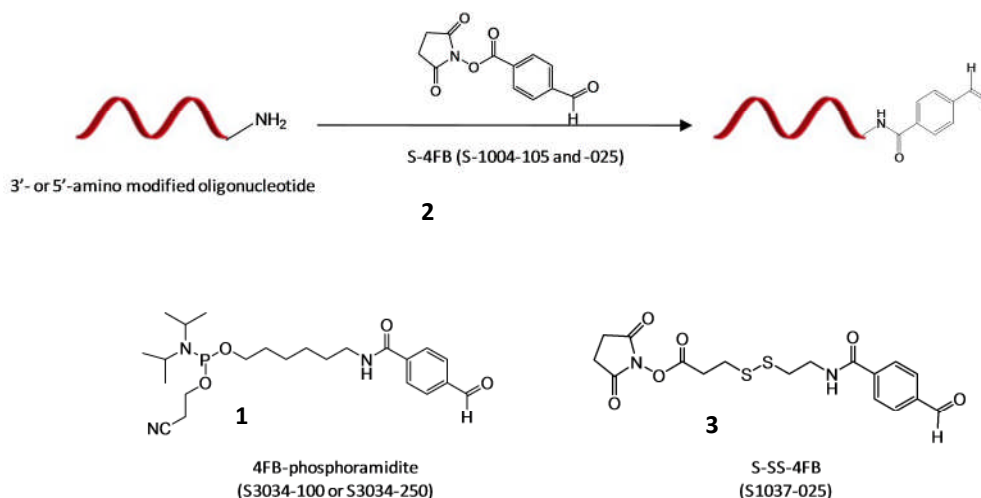


Figure 2: Schematic representation of the conversion of an amino-modified oligonucleotide to a 4FB-oligonucleotide with S-4FB (**2**) (top) and structures of 4FB-phosphoramidite (**1**) and S-SS-4FB (**3**), the reagent used to convert an amino-oligonucleotide to a 4FB-SS-oligonucleotide.

IV. The Keys to Successful Conjugation

The following are the three crucial requirements that must be fulfilled for a reproducibly successful preparation of a protein/oligonucleotide conjugate using Solulink's bioconjugation technology:

1. **Desalting:** Prior to modification, the starting protein must be thoroughly desalted, removing all amine contaminants, and exchanged into Modification Buffer, pH 7.4.
2. **Antibody concentration:** The recommended concentration of the antibody must be adhered to in all steps.
3. **Molar substitution ratio:** The Molar ratio of the Hynic on the protein and the 4FB on the oligo must be determined and within the desired range before continuing to the next step.

V. Kit Components

Component	Size	Storage ¹
S-HyNic	2 X 1.0 mg	Desiccated
S-4FB	2 X 1.0 mg	Desiccated
10X Modification Buffer ²	1.5 mL	4°C
10X Conjugation Buffer ³	1.5 mL	4°C
10X TurboLink Catalyst Buffer ⁴	1.0 mL	4°C
7K MWCO 0.5 mL Zeba Columns	4	4°C
VivaSpin 5K MWCO diafiltration	4	Room temperature
Anhydrous DMF	1 mL	Desiccated
2-Hydrazinopyridine.2HCl	25 mg	Room temperature
2-Sulfobenzaldehyde	25 mg	Room temperature
10X MES Buffer ⁵	1.5 mL	Room temperature
7K MWCO 2 mL Zeba Columns	2	4°C
10X PBS	1.5 mL	4°C

NOTES:

- 1) For convenience all kit components can be stored at 4°C-
 - a. If precipitates are present in buffers on storage at 4°C redissolve by warming to 37°C before using
- 2) 10X Modification Buffer: 1.0 M phosphate, 1.5 M NaCl, pH 7.4
- 3) 10X Conjugation Buffer: 1.0 M phosphate, 1.5 M NaCl, pH 6.0
- 4) 10X TurboLink Catalyst Buffer: 100 mM aniline, 100 mM phosphate, 150 mM NaCl, pH 6.0
- 5) 10X MES Buffer: 1.0 M MES, pH 6.0

VI. Equipment/Reagents Required But Not Provided

Variable-speed bench-top microcentrifuge
Spectrophotometer or Plate Reader
1.5 mL microcentrifuge tubes
Pipettors
Protein concentration assay reagents such as BCA or Bradford assays

VII. Protocols

a. Desalt/Buffer Exchange of the Protein

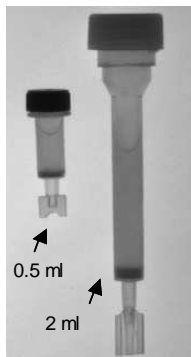


Figure 3. Zeba™ Desalt Spin Columns (0.5 and 2 ml) used to desalt starting Antibody and HyNic-modified Antibody.

Proteins/antibodies must be completely desalted into Modification Buffer (100 mM phosphate, 150 mM NaCl, pH 7.4) before modification with S-HyNic.

Any desalting method, such as dialysis, Sephadex desalting columns (NAP columns, GE Healthcare) or Zeba Desalt Spin Columns (Pierce Chemical, Cat. #89882 or 89889) can be used (Figure 2).

SoluLink recommends the use of Zeba™ Desalt Spin Columns to desalt proteins. These rapid spin columns are recommended because they do not significantly dilute the antibody during desalting.

Included in this kit are 0.5 mL Zeba Spin Desalt columns (Figure 3) that have a maximum capacity of 130 μ L. Therefore up to 1.3 mg of a 10 mg/mL solution of protein can be desalted. As this kit has been designed for two conjugations, four Zeba columns are included; one to initially desalt the antibody and one to desalt and exchange the modified antibody into conjugation buffer (see below).

Zeba Desalting Protocol

0.5ml Zeba™ Spin Column Preparation and Sample Loading

1. Remove spin column's bottom closure and loosen the top cap (do not remove cap).
2. Place spin column in a 1.5 mL microcentrifuge collection tube.
3. Centrifuge at 1,500 x g for 1 minute to remove storage solution.
4. Place a mark on the side of the column where the compacted resin is slanted upward. Place column in the microfuge with the mark facing outward in all subsequent centrifugation steps.
5. Add 300 μ L of 1x Modification Buffer (pH 7.4) or Conjugation Buffer (pH 6.0) as required to the top of the resin bed and centrifuge at 1,500 x g for 1 minute to remove buffer.
6. Repeat step 5 three additional times, discarding buffer from the collection tube.
7. Place column in a new collection tube, remove cap and slowly apply sample to the center of the compacted resin bed (30-130 μ L).
8. Centrifuge column at 1,500 x g for 2 minutes to collect desalted sample. Discard column after use.

b. Protein Modification Protocol with S-HyNic

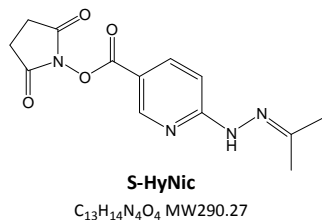


Figure 4: Structure of S-HyNic
(CAS# 362522-50-7)

	5X	10X	20X
MSR			
1 mg/ml	1.4	1.0	3.0
2.5 mg/ml	3.2	6.6	7.9
5 mg/ml	4.9	5.9	7.8

Table 1: results of a study to determine the level of HyNic incorporation on an antibody adding 5X, 10X and 20X equivalents of S-HyNic at 1.0, 2.5 and 5.0 mg/mL antibody concentration in modification buffer (100 mM phosphate, 150 mM NaCl, pH 7.2-7.4).

The modification process is the critical element of any conjugation. For this reason, we have included a more detailed discussion of this important step.

The number of functional groups incorporated per protein molecule is commonly referred to as the molar substitution ratio (MSR). The final MSR obtained after a modification reaction with S-HyNic is a function of several variables that include protein concentration, number of available amino-groups on the protein (often related to M.W.), number of excess linker equivalents added (e.g. 10X, 15X or 20X), reaction pH, and the type of heterobifunctional NHS-ester employed (e.g. S-HyNic).

In general, as the protein concentration and number of linker equivalents are increased the molar substitution ratio increases. Caution is recommended since over-modification can dramatically change the isoelectric point of the protein and result in precipitation of the protein or loss of biological activity. This is especially critical with proteins <50 kD MW.

Data has been compiled in Table 1 as an aid in determining the number of equivalents of S-HyNic required to achieve a given molar substitution ratio (MSR). In general, SoluLink recommends adding 10 molar equivalents of S-HyNic to proteins less than 100,000 daltons and adding 20 molar equivalents for proteins greater than 100,000 daltons.

Protein Modification Protocol (Calculator Worksheet 1)

- a) Dissolve a vial of pre-weighed 1.0 mg S-HyNic vial in 100 μ L anhydrous DMF
- b) Add the required volume of S-HyNic to the protein in modification buffer (100 mM phosphate, 150 mM NaCl, pH 7.4) as calculated using the [Protein-Oligonucleotide Conjugation Calculator](#). A volume that typically represents 10-20 mole equivalents of HyNic/mole protein is added and mixed thoroughly.

Notes

- I. Always maintain the percentage of DMF (vol/vol) in the final S-HyNic modification reaction at or below 5% of the total reaction volume.
 - II. PBS (**10 mM** phosphate, 150mM sodium chloride, pH 7.2) is **NOT** recommended as a modification buffer due to its poor buffering capacity. Be sure to use the SoluLink Modification Buffer for this step.
 - III. It is important to have a protein concentration at 2.5-4.0 mg/mL for efficient HyNic modification.
- c) Incubate the reaction at room temperature for 1.5 hours.
 - d) Proceed to desalt the HyNic-modified IgG into conjugation buffer (100 mM phosphate, 150 mM NaCl, pH 6.0).

It is critical that the MSR of HyNic/protein is >3.5 for all proteins larger than 100,000 daltons, and >2 for all proteins smaller than 100,000 daltons.

c. Amino Oligo modification Protocol with S-4FB (Calculator Worksheet 2)

Protocol to determine oligonucleotide concentration: OD/ μ L

- 1) dissolve X μ L oligonucleotide in 1000-X μ L nuclease free water
- 2) determine A260
- 3) OD/ μ L = A260/x

1. Desalt the oligonucleotide into nuclease free water using a 5K MWCO VivaSpin diafiltration apparatus (see appendix for further instructions). Determine oligonucleotide OD/ μ L concentration at 260 nm (see protocol at left). Oligonucleotide concentration must be 0.2-0.5 OD/ μ L for successful modification.

WARNING: Always retain the flow through following each wash. In some cases due to the length of the oligo or the membrane the oligo may flow through. The oligo can be recovered from the collected flow throughs.
2. Add 1/10 volume 10X modification buffer followed by 1/3 volume DMF.
3. Prepare a 20 mg/mL solution of S-4FB (or S-SS-4FB) in DMF.
4. Add a volume of S-4FB/DMF solution containing 20 equivalents S-4FB to the oligonucleotide solution and incubate at room temperature for 2 h.
5. Dilute with nuclease free water to 450 μ L and desalt using a second 5K MWCO VivaSpin diafiltration apparatus.
6. Wash twice with 450 μ L nuclease free water. After final desalt add 1/10 volume 10X Conjugation Buffer to the oligo solution.
7. Determine the oligonucleotide concentration at 260 nm. Oligo concentration must be 0.1-0.5 OD/ μ L for successful conjugation to protein
8. The MSR of the 4FB can be determined using the method in the appendix. The ideal MSR for oligos should be 1, though anything greater than 0.6 is acceptable for successful conjugation.

d. HyNic-Protein – 4FB-Oligo Conjugation Protocol (Calculator Worksheet 3)

1. Mix the HyNic-modified protein with the 4FB-modified oligonucleotide (2 equivalents of oligo/conjugated oligo desired)
2. Add 1/10 volume 10X TurboLink Catalyst Buffer to the conjugation solution.
3. Incubate at room temperature for 2 h
4. The conjugation reaction can be ‘visualized’ by removing an aliquot and analyzing by gel electrophoresis or spectrophotometrically on a NanoDrop spectrophotometer by determining the absorbance at A354 due to the formation of the chromophoric conjugate bond (Figure 5).

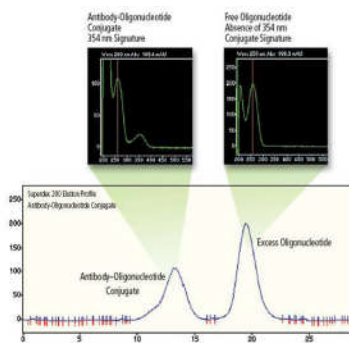


Figure 5: Purification of an antibody-20mer oligo conjugate by size exclusion HPLC (SuperDex 200; GE HealthCare). The initial peak is the desired conjugate followed by unconjugated oligonucleotide. Above each peak its UV spectrum note the 354 nm absorbance of the conjugate peak due to the formation of the chromophoric bond.

- The TurboLink Catalyst must be removed soon after the conjugation reaction is complete. The reaction may either be purified by size exclusion chromatography immediately. Or the reaction solution may be desalted using the large Zeba columns buffer exchanged in PBS for storage and later purification if necessary (see section e below).

e. Desalt of the Conjugate

2 mL Zeba™ Spin Column Preparation and Sample Loading

- Remove spin column's bottom closure and loosen the top cap (do not remove cap).
- Place spin column in a 15 mL conical collection tube.
- Centrifuge at 1,000 x g for 2 minute to remove storage solution.
- Place a mark on the side of the column where the compacted resin is slanted upward. Place column in the microfuge with the mark facing outward in all subsequent centrifugation steps.
- Add 1mL of 1x PBS (pH 7.0) as required to the top of the resin bed and centrifuge at 1,000 x g for 2 minute to remove buffer.
- Repeat step 5 three additional times, discarding buffer from the collection tube.
- Place column in a new collection tube, remove cap and slowly apply sample to the center of the compacted resin bed (200-700uL).
- Centrifuge column at 1,000 x g for 2 minutes to collect desalted sample. Discard column after use.

VIII. Kit Stability

Component	Storage	Stability
Unopened Kit	4°C	12 months after receipt
S-HyNic/supplied DMF	4°C	1 day
4FB-oligonucleotide	< -20°C	3 months
HyNic-modified protein		use immediately
Antibody-oligo conjugate following desalting without bacteriostat	4°C	4 weeks
Antibody-oligo conjugate following purification with 0.05% azide	4°C	6 months

IX. Troubleshooting

Problem	Possible Cause	Recommended Action
Poor HyNic modification of protein	- amine contaminant, e.g. Tris or glycine buffer, present in starting IgG solution	- thoroughly exchange the protein buffer by diafiltration, dialysis or desalting column before modification
	-initial protein concentration is too low	-concentrate protein using a diafiltration filter -use an initial 2-5 mg/mL for efficient labeling of proteins
Precipitation of protein on modification	- Over modification of the protein	- spin down precipitate and try to recover any protein left in solution. May require modifying new batch of protein with fewer equivalents.
Protein precipitates during conjugation reaction	-conjugation reaction pH may be close to the isoelectric point of the conjugate being formed	-conjugate at a different pH (e.g. pH 5,6,7 or higher)
Protein-DNA conjugates are degraded	-Conjugation reaction contains either single or double stranded nucleases	-Use only molecular grade water (DNase-free) when conjugating DNA to proteins

X. Appendix

a. Determining the HyNic Molar Substitution Ratio (MSR)

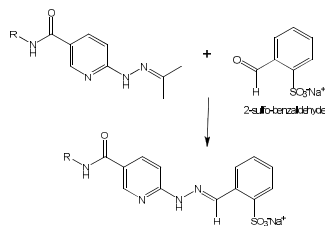


Figure 4: Scheme presenting the colorimetric assay used to quantify the number of HyNic groups on a biomolecule. The reaction product absorbs at 350 nm has a molar extinction coefficient of 18000 M^{-1} .

The determination of the number of HyNic groups/antibody is accomplished by the colorimetric assay shown in Figure 4 and described below.

HyNic MSR Quantification

Protocols to determine the MSR using a standard spectrophotometer (Method A) or a NanoDrop spectrophotometer (Method B) are described below. Both protocols begin by preparing the 2-sulfobenzaldehyde (2-sBA) Assay Reagent described in Step 1.

1. **Assay Reagent Preparation:** Prepare a 0.5 mM working solution of 2-sBA solution in 0.1 M MES buffer, pH 5.0 as follows:
 - a. Prepare a 20 mg/mL solution of 2-sulfobenzaldehyde in water.
 - b. Add 52 μL of this solution to a 15 mL conical tube containing 9.948 mL 100 mM MES Buffer (pH 5.0). Label this solution 0.5 mM 2-sBA solution.
 - c. Protect the solution from light and keep refrigerated. This solution remains stable for up to 30 days at 4°C .

Method A: Cuvette Spectrophotometer Protocol (Calculator 4)

1. Transfer 10 μL of HyNic-modified (desalted) antibody solution ($\sim 2\text{-}5\text{mg/ml}$ in 1x Conjugation Buffer) to a new 1.5 mL microfuge tube containing 490 μL 2-sBA reagent. Prepare another reaction tube (negative control) containing 490 μL 2-sBA reagent and 10 μL of 1x Conjugation Buffer.
 2. Incubate all reaction tubes at 37°C for 30 minutes or at room temp for 2 h.
 3. Remove the reaction tubes from the 37°C incubator and measure the A_{350} of both reactions using a quartz cuvette as follows:
 - a. blank the spectrophotometer at 350 nm using 500 μL 0.5mM 2-sBA solution in MES (pH 5.0) in a 1 mL quartz cuvette.
 - b. record the A_{350} of the sample and no antibody controls.
- Note-** In instances where low HyNic incorporation occurs or when antibody concentration is $< 2 \text{ mg/mL}$ the assay may require $>10 \mu\text{L}$ to achieve a detectable A_{350} reading.
4. Using the values obtained, calculate the HyNic/antibody MSR with the aid of our [Protein-Oligonucleotide Conjugation Calculator](#) or calculate the MSR by determining the hydrazone concentration using the known molar extinction

coefficient (i.e. 18,000 at 350 nm) and dividing by the known molar antibody concentration.

Method B: NanoDrop Method

1. Transfer 2 μ L of HyNic-modified (desalted) antibody solution (~2-5 mg/mL in 1x Conjugation Buffer) to a new 1.5 mL microfuge tube containing 18 μ L 2-sulfobenzaldehyde reagent. Prepare another reaction tube (negative control) containing 2 μ L 1x Conjugation Buffer reagent and 18 μ L of 2-sulfobenzaldehyde.
2. Incubate all reaction tubes at 37°C for 30 minutes or at room temp for 2 h.
3. Remove the reaction tubes from the 37°C incubator and measure the A_{350} of Determining the Molar Substitution Ratio (MSR)

Using the values obtained, calculate the HyNic/ antibody MSR with the aid of the [Protein-Oligonucleotide Conjugation Calculator](#) or calculate the MSR by determining the hydrazone concentration using the known molar extinction coefficient (i.e. 18,000 at 350 nm) and dividing by the known molar antibody concentration.

c. Vivaspin Diafiltration Protocol for Oligo desalting

NOTE- VIVASPIN 500 spin filters are made to contain and process volumes of 500 μ l or less. If volumes greater than 500 μ l are to be processed, then multiple filters or loadings may be required.

1. Open the lid of a VIVASPIN 500 filter device.
2. Transfer the oligo solution to the concentrator body of the VIVASPIN 500 filter. Bring the volume up to 500 μ L with nuclease free water.
3. Close the lid and orient the VIVASPIN 500 spin filter in the centrifuge so that the volume markers face toward the center of the centrifuge rotor each time. Use an appropriate balance tube opposite the position of the spin filter.
4. Centrifuge for 10-15 minutes @ 15,000 x g.
5. Open the filter unit, and check to make sure the volume remaining is 50 μ l or less. If the volume is greater than 50 μ l, continue to centrifuge until the volume reaches the desired level.
6. Add 450 μ l volume of nuclease free water to the oligonucleotide solution and mix it within the concentrator body using a pipette. **Do not touch or damage the filter surface with the pipette tip.**
7. Repeat steps 4 through 6 two additional times to fully desalt the oligonucleotide into nuclease free water.

NOTE- After the final spin, the oligonucleotide solution will be in a final volume of ~ 50 μ l. The entire process takes approximately 45 minutes.

11. Carefully transfer the concentrated oligonucleotide solution (~ 50 ul) from the concentrator body into a new 1.5 ml microfuge tube and measure the optical density of a 2 ul aliquot on the spectrophotometer or Nanodrop™ (OD260)

VII. References

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