

HyNic & 4FB Molar Substitution Ratio Protocol

After desalting the modified protein sample using a Zeba™ spin column, determine the protein concentration using the BCA protein assay then proceed as follows to determine the molar substitution ratio of the modified protein.

Protocol for HyNic Molar Substitution Ratio (MSR)

1. Prepare a 0.5 mM working solution of 2-sulfobenzaldehyde (2-SBA) solution in 0.1 M MES buffer, pH 5.0 as follows:
 - a) Dissolve 10 mg 2-sulfobenzaldehyde (M.W. 208.2) in 100 ul DMF.
 - b) Add 52 ul of the 2-SBA solution to a 50 ml conical tube containing 50 ml 100mM MES Buffer (pH 5.0). Label this solution 0.5 mM 2-SBA reagent.
 - c) Protect the solution from light and keep refrigerated. This solution remains stable for up to 30 days at 4°C.
2. Prepare duplicate reactions by transferring 10 ul of HyNic-modified (desalted) protein solution (2-5 mg/ml in 1X Conjugation buffer 6.0) into 1.5 ml microfuge tubes. Add 490 ul of 0.5 mM 2-SBA reagent to each sample.
3. Prepare a negative control reaction by adding 10 ul of 1X Conjugation buffer pH 6.0 to 490 ul 2-SBA reagent in a separate tube.
4. Incubate all reaction tubes at 37°C for 30 minutes.
5. Remove the reaction tubes from the 37°C incubator and
 - a) blank the spectrophotometer @ 350 nm using the negative control (1-cm quartz cuvette).
 - b) measure A_{350} of sample duplicates
6. Input the BCA measured protein concentration and the average of the A_{350} values (duplicates) into the HyNic-MSR Calculator.

NOTE-Cloudiness can sometimes appear during the MSR reaction if the protein is over-modified. This occurs due to protein aggregation/precipitation. Cloudiness is most readily observed within a quartz cuvette. It will adversely affect the accuracy of A_{350} measurements. If cloudiness appears, add of 10 ul of 10 N NaOH to the MSR reaction (and the negative blank control). This addition often clarifies the solution making for a more accurate reading, devoid of Raleigh light scattering. Always note any cloudiness that may appear in these reactions.

Protocol for 4FB Molar Substitution Ratio (MSR)

After desalting the modified protein sample using a Zeba™ spin column, determine the protein concentration using the BCA protein assay then proceed as follows to determine the molar substitution ratio of the modified protein.

1. Prepare a 0.5mM working solution of 2-hydrazinopyridine-2 HCl (2-HP) solution in 0.1 M MES

buffer, pH 5.0 as follows:

- a) Dissolve 5 mg 2-hydrazinopyridine·2HCl (M.W. 182.1) solid in 100 ul DMF.
 - b) Add 91 ul of this solution to a 50 ml conical tube containing 50 ml 100 mM MES Buffer (pH 5.0). Label this solution 0.5 mM 2-HP reagent.
 - c) Protect the solution from light and keep refrigerated. This solution remains stable for up to 30 days at 4° C. Label the solution 0.5 mM 2-HP solution.
2. Prepare duplicate reactions by transferring 10 ul of 4FB-modified (desalted) protein solution (~2-5 mg/ml in 1x Conjugation buffer pH 6.0) into two 1.5 ml microfuge tubes. Add 490 ul 2-HP reagent each to each tube.
 3. Prepare a negative control reaction by adding 10 uL of 1X Conjugation buffer pH 6.0 to 490 ul 0.5 mM 2-HP reagent in a separate tube.
 4. Incubate all reaction tubes at 37° C for 30 minutes.
 5. Remove the reaction tubes from the 37° C incubator and
 - a) blank the spectrophotometer @ 350 nm using the negative control (1-cm quartz cuvette).
 - b) measure A_{350} of sample duplicates
 7. Input the measured protein concentration and the average of the A_{350} values (duplicates) into the 4FB-MSR Calculator.

NOTE-Cloudiness can sometimes appear during the MSR reaction if the protein is over-modified. This occurs due to protein aggregation/precipitation. Cloudiness is most readily observed within a quartz cuvette. It will adversely affect the accuracy of A_{350} measurements. If cloudiness appears, add of 10 ul of 10 N NaOH to the MSR reaction (and the negative blank control). This addition often clarifies the solution making for a more accurate reading, devoid of Raleigh light scattering. Always note any cloudiness that may appear in these reactions.