

Determining the HyNic/Protein Molar Substitution Ratio (MSR)



Materials Required

Reagents

1X MES Buffer (100 mM MES, pH 5.0)

2-Sulfobenzaldehyde

Conjugation Buffer

Catalog

S-4026

S-2005

S-4002

Equipment

1.5 mL microcentrifuge tubes

Spectrophotometer or Nanodrop

Nuclease-free H₂O

The determination of the number of HyNic groups/protein is accomplished by a colorimetric assay presented in Figure 1. In the assay, 2-sulfobenzaldehyde (2-SBA) forms a chromophoric bis-arylhydrazone product with incorporated HyNic groups that absorbs at 345 nm and has a molar extinction coefficient of 28,500 M⁻¹ cm⁻¹.

HyNic Colorimetric MSR Assay Protein / Spectrophotometer Protocol

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. Weigh 10 mg of 2-sulfobenzaldehyde.
 - b. Create a 100 mg/mL solution of 2-sulfobenzaldehyde (M.W. 208.2) in nuclease-free H₂O.
 - c. Add 52 µl of the 2-SBA solution to a 50 ml conical tube containing 50 ml of 100 mM MES Buffer, pH 5.0.
 - d. Label this solution "0.5 mM 2-SBA solution."
 - e. Protect the solution from light and keep refrigerated. This solution remains stable for up to 30 days at 4° C.
2. Fill three microcentrifuge tubes with 490 µL of 0.5 mM 2-SBA solution.
 - a. Add 10 µL of 1x Conjugation Buffer, pH 6.0 to the first microcentrifuge tube, which will be the "Blank".
 - b. Add 10 µL of desalted HyNic-modified protein solution (~2-5 mg/ml in 1X Conjugation Buffer, pH 6.0) to the second and third microcentrifuge tubes to create duplicates.
 - c. Label all microcentrifuge tubes.
 - d. Reaction volumes may be proportionately decreased depending on cuvette size.
3. Incubate all reaction tubes at 37° C for 30 minutes or at room temperature for 2 hours.
4. Remove the reaction tubes from the 37° C incubator and measure the A₃₄₅ of the reaction using a quartz cuvette as follows:
 - a. Add the whole volume of the "Blank" microcentrifuge tube to a 1-cm cuvette and blank the spectrophotometer at 345 nm.
 - b. Measure the A₃₄₅ of the duplicates. Use the average of the values.

Note- In instances where low HyNic incorporation occurs or when protein concentration is < 2 mg/mL the assay may require > 10 µL to achieve a detectable A₃₄₅ reading.
5. Using the averaged values obtained, calculate the HyNic/protein MSR with the aid of our [MSR Calculator](#) or calculate the MSR by determining the hydrazone concentration using the known molar extinction coefficient (i.e. 28,500 M⁻¹ cm⁻¹ at 345 nm) and dividing by the known molar protein concentration.

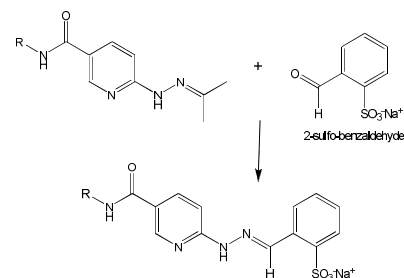


Figure 1: Scheme presenting the colorimetric assay used to quantify the number of HyNic groups on a biomolecule. The bis-arylhydrazone product absorbs at 345 nm has a molar extinction coefficient of 28,500 M⁻¹ cm⁻¹.