

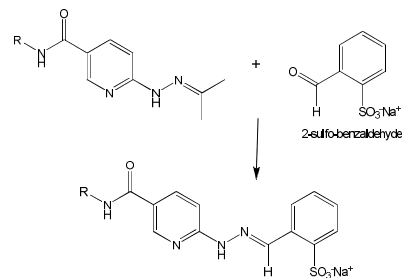
# Determining the HyNic/Oligo Molar Substitution Ratio (MSR)



## Materials Required

Reagents	Catalog #	Equipment
1X MES Buffer (100 mM MES, pH 5.0)	S-4026	1.5 mL microcentrifuge tubes
2-Sulfobenzaldehyde	S-2005	Spectrophotometer or Nanodrop
Conjugation Buffer	S-4002	Nuclease-free H <sub>2</sub> O

The determination of the number of HyNic groups/oligonucleotide is accomplished by a colorimetric assay presented in Figure 1 using a spectrophotometer. 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<sup>-1</sup> cm<sup>-1</sup>.



**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<sup>-1</sup> cm<sup>-1</sup>.

## HyNic Colorimetric MSR Assay Oligonucleotides / 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 H<sub>2</sub>O.
  - c. Add 52  $\mu$ l 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  $\mu$ L of 0.5 mM 2-SBA solution.
  - a. Add 10  $\mu$ L of 1x Conjugation Buffer, pH 6.0 to the first microcentrifuge tube, which will be the "Blank".
  - b. Add 10  $\mu$ L of HyNic-modified oligonucleotide 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 the microcentrifuge tubes at 37° C for 30 minutes.
4. Add the whole volume of the "Blank" microcentrifuge tube to a 1-cm cuvette and blank the spectrophotometer at 345 nm.
5. Measure the A<sub>345</sub> of the duplicates.
6. Input the average A<sub>345</sub> value of the duplicates into the [HyNic/Oligonucleotide MSR calculator](#).
7. If the value is between 0.8 and 1.2, then the oligonucleotide is modified and desalted.
  - a. If the value is below 0.8, the amino incorporation during oligonucleotide solid phase synthesis was inefficient or the modification reaction with S-HyNic did not succeed.
  - b. If the value is above 1.2, then there is most likely excess HyNic in solution. Use a Vivaspin 500 to desalt the excess HyNic.