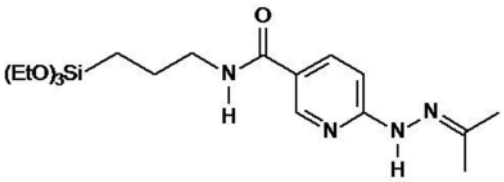
HyNic Silane

$C_{18}H_{22}N_4O_4Si$, MW: 396.4
Catalog# HL-1002

HyNic Silane Coating of Silica Surface Protocol



Silica Bead Modification Protocol

1) Weigh out 100 mg of silica beads.
2) Wash the silica beads 3 times with EtOH; pellet the bead on a centrifuge for 2 minutes at 750g, remove the supernatant. Bring the beads up in EtOH and repeat.
3) Make a 2% solution of HyNic Silane (10 mg) in EtOH (500 µL), add 2% (10 µL) water to the solution to dissolve any remaining solids. This may require intensive vortexing to get the silane into solution.
4) Add the HyNic Silane solution to the washed bead pellet so the silane/silica ratio is 20% w/v (500 µL).
5) Vortex the bead sample and incubate at room temperature on a rotator for 30 minutes.

Note: Check the pH periodically with pH paper and be sure that it doesn't go below pH 7.4 during the incubation steps!! Adjust the pH with 1M NaOH, If necessary.

6) Add an additional 2% (10 µL) water to the bead solution and continue the incubation for 15 minutes.
7) Add additional 10% (50 µL) water to the bead solution and continue the incubation for 5 mintues.
8) The washing step is very important: Washed the beads 3X each with water, ethanol, water, PBS and Conjugation Buffer (100 mM sodium phosphate, 150mM NaCl, pH 6.0) in that order, using the spin protocol from step 1.
9) Check the supernatant to see if there is a significant A310 from the HyNic Silane; add 100 µL of supernatant to 900 µL of Buffer. The A310 should give a reading no higher than 0.05.
10) Bring the beads up in Conjugation Buffer such that the solution is a 20% w/v beads in Conjugation Buffer.

The HyNic-modified beads are now ready to be conjugated the 4FB-modified biomolecule.

Note 1: For large biomolecules (proteins and antibodies), 20µg of protein/mg of bead is recommended for maximum conjugation. Allow the HyNic beads to conjugate with the protein in the presence of TurboLink Catalyst Buffer for 4 hours at room temperature on a rotor or shaker.

Note 2: The HyNic functional group is only stable for a few weeks on the glass surface. For best results, the HyNic-modified beads should be reacted right away with the 4FB-modified biomolecule.

Note: This protocol can be downloaded from the appropriate category in the Solulink Library at http://www.solulink.com/library.

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