

**SiaRich™  $\alpha$ 2,3-Specific Resin Kit contents**

| Catalog # | Description                          | Size      | Storage             | Binding Capacity             |
|-----------|--------------------------------------|-----------|---------------------|------------------------------|
| SR2301    | SiaRich™ $\alpha$ 2,3-Specific Resin | 2 mL      | 4°C, up to 6 months | 300 $\mu$ g of bovine fetuin |
| BA0102    | 5X SiaFind™ Binding Buffer 2         | 2x 100 mL | 4 to 25°C           |                              |

*Above products are for research use only and not for resale or for any use in the manufacture of a therapeutic or for any diagnostic purpose.*

**Product Description**

SiaFind™  $\alpha$ 2,3-Specific Lectenz® is a sialic acid affinity reagent for the detection, separation, and enrichment of sialoglycans terminating in Neu5Ac $\alpha$ 2,3Gal commonly found in glycoconjugates (glycoproteins, glycolipids, and oligo- or polysaccharides). It has high affinity and specificity towards  $\alpha$ 2,3 linked sialic acids on glycans. However, it does not bind effectively to branched sialylated epitopes such as sialyl Lewis A/X. This reagent can be employed as a capture reagent in a variety of applications.

**SiaRich™  $\alpha$ 2,3-Specific Resin** (Cat #SR2301) is an agarose resin coupled with the SiaFind™  $\alpha$ 2,3-Specific Lectenz® and is designed for the rapid and specific enrichment of sialoglycoconjugates terminating in Neu5Ac $\alpha$ 2,3Gal from a complex sample. Specifically bound  $\alpha$ 2,3-sialylated glycoconjugates are non-competitively eluted from the resin for downstream analysis such as mass spectrometry, ELISA, and Western blotting.

**Form, Storage, and Use**

The **SiaRich™  $\alpha$ 2,3-Specific Resin** is supplied as a 50% slurry in Tris Buffered Saline ready for affinity chromatography. The kit includes 2 bottles of 5X Binding Buffer 2 (Cat #BA0102) for equilibration, binding, washing, and storage.

**Affinity Chromatography Guide**

Affinity chromatography may be performed in a disposable mini column.

Prepare 1X Binding Buffer 2 (0.025 M EPPS, 0.1 M NaCl, pH 7.5), Elution Buffer (0.025 M EPPS, 2.1 M NaCl, pH 7.5) and Regeneration Buffer (0.025 M EPPS, 0.1 M NaCl, 5 M guanidine-HCl, pH 7.5) according to the following table (analytical grade sodium chloride and guanidine hydrochloride supplied by the customer).

| Buffer                             | 5X Binding Buffer 2 | Sodium Chloride (NaCl; MW=58.44) | Guanidine Hydrochloride (CH <sub>5</sub> N <sub>3</sub> ·HCl; MW=95.53) | Ultra-pure Water (mL) |
|------------------------------------|---------------------|----------------------------------|-------------------------------------------------------------------------|-----------------------|
| <b>1X Binding Buffer 2</b> (50 mL) | 10 mL               |                                  |                                                                         | 40 mL                 |
| <b>Elution Buffer</b> (50 mL)      | 10 mL               | 5.84 g                           |                                                                         | 38 mL                 |
| <b>Regeneration Buffer</b> (50 mL) | 10 mL               |                                  | 23.88 g                                                                 | 19 mL                 |

- Transfer 1 mL of fully resuspended SiaRich™  $\alpha$ 2,3-Specific Resin into a mini-column for a packed resin volume of 500  $\mu$ L.
- Equilibrate the resin with 5 resin volumes of 1X Binding Buffer 2.
- Load 100 to 200  $\mu$ L sample prepared in 1X Binding Buffer 2. Collect flow-through if desired.
- Wash with 5-10 resin volumes of 1X Binding Buffer 2. Collect flow-through if desired.
- Elute bound analyte(s) with 5-10 resin volumes of Elution Buffer and/or Regeneration Buffer. Collect elution in small fractions if desired, for example, 0.25 to 1 mL per fraction.

*Note: Tightly bound  $\alpha$ 2,3-sialylated glycoconjugates may require the use of Regeneration Buffer to be eluted from the resin.*

- Regenerate the resin with 5 resin volumes of Regeneration Buffer.
- Rinse the resin with 5 resin volumes of 1X Binding Buffer 2, cap the tip of the column, then add another resin volume for storage. (Optional: For long-term storage, add ethanol to 20%.)
- Seal and store the column at 4°C. The column may be reused several times if regenerated and stored properly.

**Additional considerations:**

For peptide enrichment for mass spectrometry, the SiaRich™  $\alpha$ 2,3-Specific Resin affinity chromatography fractions may be loaded directly onto a C18 SPE column for desalting, buffer exchange, and concentration.