SiaRich™ α2,3-Specific Column Kit Contents

Catalog #	Description	Size	Storage	Binding capacity
SC2301	SiaRich™ α2,3-Specific Affinity Column	1 mL	4°C	300 µg fetuin or equivalent
BA0103	5X SiaFind™ Binding Buffer 3 (SBB3)	3x 100 mL	4 to 25°C	
BA0301	5X SiaFind™ Regeneration Buffer 1 (SRB1)	1x 100 mL	4 to 25°C	

This product is 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™ α2,3-Specific Lectenz® is a sialic acid affinity reagent for the detection, separation and enrichment of sialoglycans terminating in Neu5Acα2,3Gal commonly found in glycoconjugates (glycoproteins, glycolipids, and oligo- or polysaccharides). It has high affinity and specificity towards α2,3 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.

The **SiaRich™ α2,3-Specific Column** (Cat #SC2301) is a SiaFind™ α2,3-Specific Lectenz®-coupled column for rapid enrichment and purification of α2,3-linked sialoglycoconjugates by affinity chromatography. Specifically bound sialylated glycoproteins or other biomolecules may be eluted competitively or non-competitively from the column.

Form and Storage

The SiaRich™ affinity column is prepacked and FPLC-ready. It is supplied in SiaFind™ Binding Buffer 3 (25 mM EPPS, 110 mM NaCl, pH 7.5). It is stable at 4°C for three months from the date of production. Never freeze the column.

All 5X buffers should be diluted to 1X with ultrapure water. For instance, to make 250 mL, add 50 mL of any 5X buffer to 200 mL water and mix by inversion. All buffers may be stored at 4 to 25°C.

Affinity Chromatography Guide

Affinity chromatography may be performed using the SiaRich™ α2,3-Specific Column on an FPLC system or manually by syringe injection. Prepare 1X SiaFind™ Binding Buffer 3 from the 5X binding buffer (Cat #BA0103). Prepare 1X SiaFind™ Regeneration Buffer 1 (25 mM EPPS, 1 M NaCl, pH 7.5) from the 5X regeneration buffer (Cat #BA0301).

1. Equilibrate column with 5 mL SBB3 at 1 mL/min.
2. Inject an analyte at 0.5 mL/min. For the first run, 400 µg fetuin in 1 mL SBB3 is recommended.
3. Wash column with 10 mL SBB3 at 1 mL/min.
4. Elute bound analyte(s) non-competitively with 5 mL of SRB1 or competitively with 5 mL of 50 mM 3'-sialyllactose dissolved in SBB3 at 1 mL/min.
   Note: Impurities in 3'-sialyllactose may lead to significant absorption at 280 nm, artificially increasing the elution peak. SDS-PAGE analysis of elution fractions may be required to identify the enriched fractions.
5. Regenerate column with 5 mL of SRB1 at 1 mL/min.
6. Repeat step 1 to prepare for the next run or for short-term storage of the column.
7. Optional: For long-term storage, fill the column with 5 mL SBB3 in 20% ethanol (1:3:1 mixture of 5X SBB3:H_2O:ethanol).
8. The column may be reused until the elution peak contains < 300 µg of 400 µg fetuin injected as the sole analyte.

Notes:

1. Complex samples, e.g., cellular extracts, may be injected at a slower flow rate to maximize binding.
2. Concentrated samples must be well-diluted in SBB3 to ensure optimal binding to the column.
3. Perform downstream analysis, such as Western Blot using our SiaFind™ α2,3-Specific Lectenz®, to confirm enrichment of α2,3-linked sialoglycoconjugates.

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