_N_-GlyRich™ Core-Specific Resin Kit contents

Catalog #	Description	Size	Storage	Binding Capacity
NR0101	*N*-GlyRich™ Core-Specific Resin	2 mL	4°C, up to 6 months	200 µg Ribonuclease B peptides
BA0104	5X Tris Buffered Saline	100 mL	4 to 25°C	
BA0202	5X Elution Buffer 2	100 mL	4 to 25°C	
GC0201	Ribonuclease B peptides	50 µg	4°C, up to 6 months	

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

N-GlyFind™ Core-Specific Reagent is a recombinant protein engineered from *Mus musculus* Fbs1 (Fbxo2), a component of the E3 ubiquitin ligase complex. This highly purified affinity reagent is designed for sensitive, robust, and specific detection of ManGlcNAc2-Asn within the *N*-glycan core structure commonly found in glycoproteins.

***N*-GlyRich™ Core-Specific Resin** (Cat #NR0101) is an agarose resin coupled with the *N*-GlyFind™ Core-Specific Reagent and is designed for the rapid and specific enrichment of *N*-glycoproteins and *N*-glycopeptides from a complex sample. Specifically bound *N*-glycoconjugates are non-competitively eluted from the resin for downstream analysis such as mass spectrometry, ELISA, and Western blotting.

Form, Storage, and Use

The ***N*-GlyFind™ Core-Specific Resin** is supplied as a 50% suspension in Tris Buffered Saline ready for affinity chromatography. The kit includes 5X Tris Buffered Saline (Cat #BA0104) for binding, washing, and storage; 5X Elution Buffer 2 (Cat #BA0202); and endoproteinase GluC-digested Ribonuclease B (Cat #GC0201) as an analyte control.

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.

The Ribonuclease B peptides should be reconstituted in 50 µL molecular grade water to make a solution of 1.0 mg/mL in GluC digestion buffer (0.1 M NaCl, 50 mM Tris-HCl, 0.5 mM Glu-Glu, pH 8.0). An amount of 10 µg may be used as a control analyte. Non-glycosylated peptides should flow through, while *N*-glycopeptides should bind and be subsequently eluted.

Affinity Chromatography Guide

Affinity chromatography may be performed in a disposable mini column. Prepare Binding Buffer from 5X Tris Buffered Saline. Prepare 1X Elution Buffer 2 (0.2 M Glycine-HCl, 20 mM NaCl, pH 3.5) from 5X Elution Buffer 2. Prepare Regeneration Buffer (5 M guanidine HCl in Tris Buffered Saline). For example, for 10 mL, dissolve 4.78 g of guanidine HCl in 5.7 mL of ultrapure water and 2 mL of 5X Tris Buffered Saline.

1. Transfer 1000 µL of the resuspended *N*-GlyRich™ Core-Specific Resin in a mini-column for a packed resin volume of 500 µL.
2. Equilibrate the resin with 5X resin volumes of Binding Buffer.
3. Load 100 to 200 µL sample prepared in Binding Buffer. Collect flow-through.
4. Wash with 5X resin volumes of Binding Buffer. Collect flow-through.
5. Elute bound analyte(s) with 5X resin volumes of 1X Elution Buffer 2 and/or Regeneration Buffer. Collect elution in small fractions if desired, for example, 0.1 to 0.5 mL per fraction.

   Note: *N*-glycopeptides and glycoproteins lightly *N*-glycosylated can be eluted with 1X Elution Buffer 2 alone. Heavily *N*-glycosylated glycoproteins may require the use of Regeneration Buffer to be released from the resin.
6. Regenerate the resin with 5X resin volumes of Regeneration Buffer.
7. Rinse the resin with 1 resin volume of Binding Buffer, cap the tip of the column, then add another resin volume for storage. (Optional: For long-term storage, add ethanol to 20%.)
8. Seal and store the column at 4°C. The column may be reused several times if regenerated and stored properly.

Additional considerations:

1. For peptide enrichment for mass spectrometry analysis, the *N*-GlyRich™ affinity chromatography elution fractions may be loaded directly onto a C18 SPE column for desalting, buffer exchange, and concentration.

Corporate Headquarters: Innovation Gateway, 111 Riverbend Rd, Athens, GA 30602, USA
Satellite Operations: San Diego Science Center, 3030 Bunker Hill St, San Diego, CA 92109, USA
Phone: +1 (706) 549-4484 E-mail: sales@lectenz.com Web: https://www.lectenz.com