

N-GlyFind™ Core-Specific Reagent Kit contents

Catalog #	Description	Size	M. W.	Storage
NK0101	N-GlyFind™ Core-Specific Reagent (NP0102-1MG)	1 mg, lyophilized	25,900	-20°C, up to 12 months
	5X Tris Buffered Saline (BA0104)	100 mL		4 to 25°C
NK0101B	N-GlyFind™ Core-Specific Reagent Biotinylated (NP0102B-1MG)	1 mg, lyophilized	26,400	-20°C, up to 12 months
	5X Tris Buffered Saline (BA0104)	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

N-GlyFind™ Core-Specific Reagent Kits (NK0101 and NK0101B) contain 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. Applications include use as a primary probe in ELISA, Western blot, and other assays. Each kit also includes 5X Tris Buffered Saline (BA0104) as a binding buffer to ensure maximum reagent specificity and ease of use.

Each **N-GlyFind™ Core-Specific Reagent** has a molecular mass of about 26 kD and works without bivalent metal ions. It is 8xHis-tagged at its *N*-terminus, and an anti-polyhistidine antibody or, in the case of the biotinylated version, a streptavidin conjugate can be used for detection. The 8xHis tag may be removed by FasTEV™ (Cat #GE0501), a TEV protease with enhanced stability and catalytic activity.

Form and Storage

The **N-GlyFind™** reagents are supplied lyophilized in a storage buffer (50 mM EPPS, 200 mM NaCl, pH 7.5) and should be reconstituted in 100 µL molecular water grade to yield a 10 mg/mL solution. Concentration is determined by spectrophotometry using E^{1%} 26.4. Once reconstituted, store at 4°C for up to 5 days or -20°C for up to 6 months. Aliquoting is recommended to avoid repeated freeze-thaw cycles.

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.

Western Blotting Guide

Use 0.1 - 1.0 µg fetuin and/or RNase B as positive control.

Prepare 1X TBS (25 mM Tris-HCl, 2.7 mM KCl, 137 mM NaCl, pH 7.4) from the 5X Tris Buffered Saline (Cat #BA0104). Prepare TBS plus 0.1% Tween-20 (TBST) for membrane washing.

Prepare TBST with 5% globulin-free BSA for blocking. Incubate the membrane at room temperature for 1 h with agitation.

Prepare the **N-GlyFind™ Core-Specific Reagent** in TBST with 0.5% globulin-free BSA: 26 µg/mL of the native reagent (Cat #NP0102) or 2.6 µg/mL of the biotinylated reagent (Cat #NP0102B). Incubate at room temperature for 1 h with agitation. Rinse membrane 3 X 5 min with TBST.

Incubate with a 2° probe diluted in TBST with 0.5% globulin-free BSA, e.g., a 10,000 dilution of an anti-polyhistidine tag antibody-HRP conjugate for the native reagent (Cat #NP0102) or 1 µg/mL of a streptavidin-HRP solution for the biotinylated reagent (Cat #NP0102B). Rinse membrane 3 X 5 min with TBST.

Rinse membrane 3 X 5 min with TBS before applying HRP chemiluminescent substrate for detection.