_N_-GlyFind™ Core-Specific Reagent SureLight® 488 Kit contents

Catalog #	Description	Size	M. W.	Storage
NK0101F	*N*-GlyFind™ Core-Specific Reagent SureLight® 488 (NP0102F-1MG)	1 mg, lyophilized	26,500	-20°C, up to 6 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 SureLight® 488 Kit** (NK0101F) contains 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 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™** 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 can be used for detection or, in the case of the fluorescent version, directly detected. The 8xHis tag may be removed by FasTEV™ (Cat #GE0501), a TEV protease with enhanced stability and catalytic activity.

***N*-GlyFind™ Core-Specific Lectenz® SureLight® 488** is made for fluorescence detection (Ex 496 nm; Em 517 nm). Applications include Western Blot, FLISA, flow cytometry, etc.

Form and Storage

The ***N*-GlyFind™ SureLight® 488** reagent is 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. The actual absorbance at 280 nm is calculated by measuring the observed absorbance at 280 nm and subtracting the absorbance at 493 nm multiplied by a correction factor of 0.11; $A280_{actual} = A280_{observed} - (A493 \times 0.11)$. Store at 4°C for up to 15 days or -20°C for up to 6 months protected from light. **Aliquoting is recommended to avoid repeated freeze-thaw cycles.** Store in dark tubes, and perform experiments protected from light.

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 0.5 - 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 26 µg/mL ***N*-GlyFind™ Core-Specific Reagent SureLight® 488** in TBST with 0.5% globulin-free BSA

Incubate membrane in the probe solution at room temperature for 1 h with agitation protected from light. Rinse membrane 3 X 5 min with TBST.

Rinse membrane 3 X 5 min with TBST. Detect fluorescence using an appropriate imaging system.

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