

### SiaFind™ $\alpha$ 2,6-Specific Reagent Kit contents

Catalog #	Description	Size	M. W.	Storage
SK2601	SiaFind™ $\alpha$ 2,6-Specific Reagent (SP2602-100UG)	100 $\mu$ g, lyophilized	34,000	-20°C, up to 6 months
	5X SiaFind™ Binding Buffer 2 (BA0102)	100 mL		4 to 25°C
SK2601B	SiaFind™ $\alpha$ 2,6-Specific Reagent, Biotinylated (SP2602B-100UG)	100 $\mu$ g, lyophilized	34,500	-20°C, up to 6 months
	5X SiaFind™ Binding Buffer 2 (BA0102)	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™  $\alpha$ 2,6-Specific Reagent Kits** (SK2601 and SK2601B) contain a recombinant protein engineered from *Polyporus squamosus* lectin (PSL). This highly purified affinity reagent is designed for sensitive, robust, and specific detection of Sia $\alpha$ 2,6Gal commonly found in glycoconjugates (glycoproteins, glycolipids, and oligo- or polysaccharides). Applications include use as a primary probe in ELISA, Western blot, and immunohistochemistry. It is not recommended for flow cytometry. Each kit also includes a 5X binding buffer to ensure maximum reagent specificity and ease of use.

This product does not contain any detectable activities of proteases or glycosidases.

Each **SiaFind™  $\alpha$ 2,6-Specific Reagent** has a molecular mass of about 34 kD and works with or 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 **SiaFind™** reagents are supplied lyophilized in a storage buffer (50 mM EPPS, 200 mM NaCl, pH 7.5) and should be reconstituted in 100  $\mu$ L molecular water grade to yield a 1 mg/mL solution. Concentration is determined by spectrophotometry using E<sup>1%</sup> 22.0. 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  $\mu$ g fetuin and/or 6'-sialyllactose-BSA as positive control.

Prepare 1X SiaFind™ Binding Buffer 2 (SBB2, 25 mM EPPS, 100 mM NaCl, pH 7.5) from the 5X binding buffer (Cat #BA0102). Prepare SBB2 plus 0.1% Tween-20 (SBB2T) for membrane washing.

Prepare SBB2T with 5% globulin-free BSA for blocking. Incubate the membrane at room temperature for 1 h with agitation.

Prepare the **SiaFind™  $\alpha$ 2,6-Specific Reagent** in SBB2T with 0.5% globulin-free BSA: 0.5  $\mu$ g/mL of the native reagent (Cat #SP2602) or 0.2  $\mu$ g/mL of the biotinylated reagent (Cat #SP2602B). Incubate at room temperature for 1 h with agitation. Rinse membrane 3 X 5 min with SBB2T.

Incubate with a 2° probe diluted in SBB2T 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 #SP2602) or 1  $\mu$ g/mL of a streptavidin-HRP solution for the biotinylated reagent (Cat #SP2602B). Rinse membrane 3 X 5 min with SBB2T.

Rinse membrane 3 X 5 min with SBB2 before applying HRP chemiluminescent substrate for detection.

**Note:** Common buffers, e.g., TBS and PBS, may be used as binding buffer without significant loss of binding signal.