



# $\alpha(1-2)$ FUCOSIDASE

## SPECIFICATIONS

**Product Code:** GK80170  
**Specific Activity:**  $\geq 400$  mU/mg  
**Activity:**  $\geq 100$  mU/ml  
Shipped with cold pack for next day delivery.  
Store at 4°C. **DO NOT FREEZE**  
**Formulation:** A sterile-filtered solution in 20 mM Tris-HCl, 50 mM NaCl, 0.1 mM EDTA, 0.006% sodium azide, and 100  $\mu$ g/mL BSA (pH 7.5)  
**Stability:** Stable at least 12 months when stored properly. Several days exposure to ambient temperatures will not reduce activity.

Glyko<sup>®</sup> sequencing-grade  $\alpha(1-2)$  Fucosidase ( $\alpha$ -L-fucoside fucohydrolase, EC 3.2.1.51) cleaves non-reducing terminal fucose, linked  $\alpha(1-2)$  to the galactose of terminal Gal-GlcNAc disaccharide structures. The presence of fucose linked to the N-acetylglucosamine will block cleavage. Substrates for  $\alpha(1-2)$  Fucosidase are shown in Figure 1.

Glyko  $\alpha(1-2)$  Fucosidase is isolated from *Xanthomonas* sp.

Glyko  $\alpha(1-2)$  Fucosidase is useful for:

- Determining fucose linkages
- Deglycosylating glycoproteins with Lewis structures

## PRODUCT DESCRIPTION

**Supplied Reagents** (research pack only)

- 5x Reaction Buffer 5.0  
(50 mM sodium phosphate, pH 5.0)

**Purity:** Each lot of Glyko  $\alpha(1-2)$  Fucosidase is tested for contaminating activities by incubating the enzyme at 37°C for 24 hours with the appropriate substrates; the detection limit of these assays is 5  $\mu$ U/ml (IUB). A passing lot will have no detectable activity.

Contaminant	Substrate
$\beta$ -N-acetylglucosaminidase	<i>p</i> -nitrophenyl- $\beta$ -D-N-acetylglucosaminide
$\alpha$ -galactosidase	<i>p</i> -nitrophenyl- $\alpha$ -D-galactopyranoside
$\beta$ -galactosidase	<i>p</i> -nitrophenyl- $\beta$ -D-galactopyranoside
neuraminidase	methylumbelliferyl- $\alpha$ -D-N-acetylneuraminic acid
$\alpha$ -mannosidase	<i>p</i> -nitrophenyl- $\alpha$ -D-mannopyranoside
$\beta$ -mannosidase	<i>p</i> -nitrophenyl- $\beta$ -D-mannopyranoside
$\alpha(1-3,4)$ fucosidase	methylumbelliferyl-Lewis X trisaccharide*
$\beta$ -xylosidase	4-methylumbelliferyl-7- $\beta$ -D-xylopyranoside

\* Lewis X trisaccharide is Gal  $\beta(1-4)$ [Fuc  $\alpha(1-3)$ ]GlcNAc

To test for contaminating protease, 10  $\mu\text{g}$  of denatured BSA is incubated at 37°C for 24 hours with 2  $\mu\text{l}$  of enzyme. SDS-PAGE analysis of the treated BSA shows no evidence of degradation.

**Specificity:** Non-reducing terminal fucose when linked  $\alpha(1-2)$  to galactose of a Gal-GlcNAc disaccharide structure. The presence of fucose linked to the N-acetylglucosamine will block cleavage.

**Molecular Weight:** 85,000 daltons

## ASSAY

One unit of Glyko  $\alpha(1-2)$  Fucosidase is defined as the amount of enzyme required to catalyze the release of 1  $\mu\text{mole}$  of fucose from 2'-fucosyl-lactose per minute at pH 5.5 and 37°C.

Note: *p*-nitrophenyl- $\alpha$ -L-fucopyranoside is not a suitable substrate for this enzyme.

**Additional Reagents** (not supplied)

- 250  $\mu\text{M}$  4-methylumbelliferyl-2-O-( $\alpha$ -L-fucopyranosyl)- $\beta$ -D-galactopyranoside (Toronto Research Chemicals #M333030) in 1x Reaction Buffer 5.0 (50 mM sodium phosphate, pH 5.0)
- 0.6 M sodium carbonate
- BG13  $\beta(1-4)$  Galactosidase

### Procedure

1. Add 20  $\mu\text{l}$  of substrate solution to 2 tubes and pre-warm to 37°C.
2. Add 1  $\mu\text{l}$  of enzyme solution (or dilution) to one tube and mix.
3. Incubate both tubes for 15 minutes at 37°C.

4. Stop the reactions by placing the tubes in a 100°C water bath for 5 minutes.
5. Cool to 37°C and add 1.5 mU of GE12  $\beta(1-4)$  Galactosidase to each tube.
6. Incubate for 10 minutes at 37°C.
7. Add 1 ml of 0.6 M sodium carbonate to each tube.
8. Read fluorescence in a spectrofluorometer using excitation and emission wavelengths of 390 and 450 nm, respectively.

Make a standard curve of methylumbelliferone (MU) in 0.6 M sodium carbonate.

### Calculation

$$\text{Units/ml} = (\text{nmol MU}_{\text{sample}} - \text{nmol MU}_{\text{blank}}) \times D \div 15$$

where D = Dilution factor

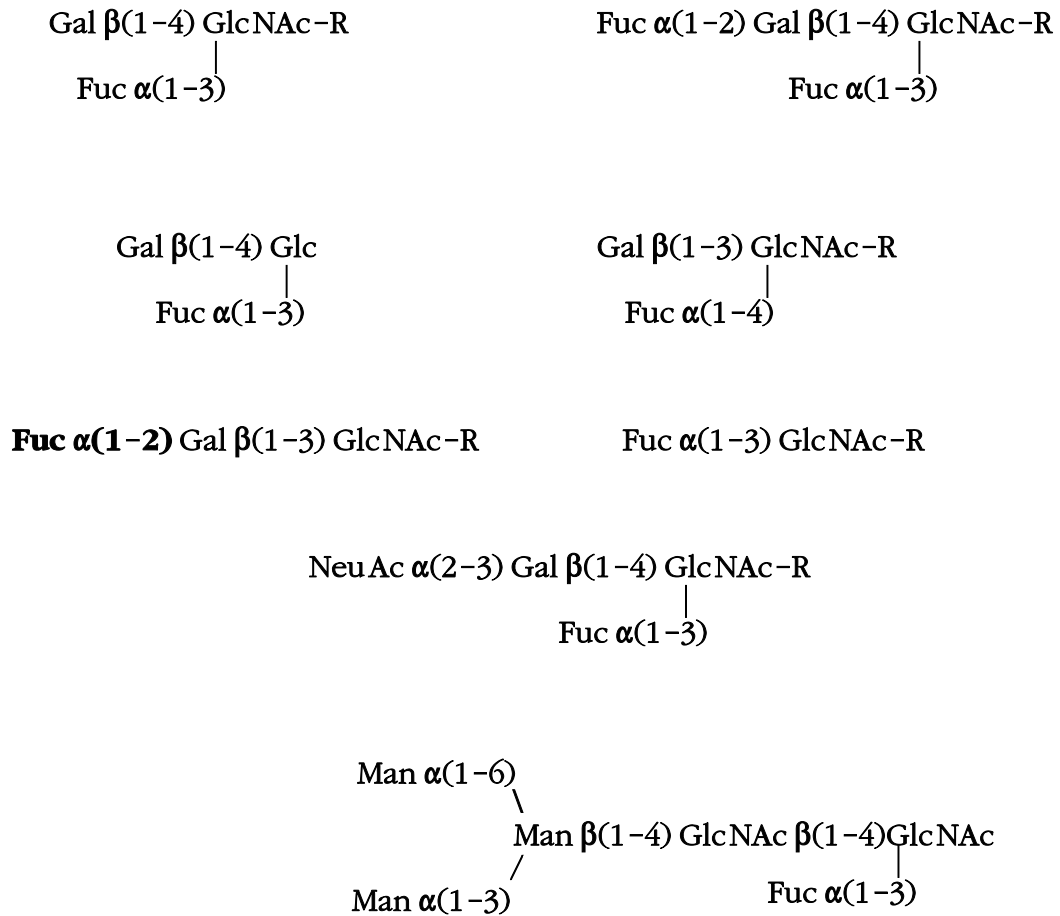
## SUGGESTIONS FOR USE

### Procedure for De-fucosylation

1. Add up to 1 nmole of oligosaccharide to a tube.
2. Add de-ionized water to a total of 15  $\mu\text{l}$ .
3. Add 4  $\mu\text{l}$  of 5x Reaction Buffer 5.0.
4. Add 1  $\mu\text{l}$  of  $\alpha(1-2)$  Fucosidase.
5. Incubate for 1 hour at 37°C.

**Figure 1 - Fucose residues cleaved by  $\alpha(1-2)$  Fucosidase (shown in bold)**

Man - mannose; Gal - galactose; Fuc - fucose; GlcNAc - N-acetylglucosamine;  
NeuAc - N-acetylneuraminic acid (sialic acid); R - residue





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