

## O-Glycanase from *Streptococcus pneumoniae*, Recombinant

Cat. No. NATE-0496

Lot. No. (See product label)

### Introduction

**Synonyms** O-Glycanase

### Product Information

**Species** *Streptococcus pneumoniae*

**Source** *E. coli*

**Form** A sterile-filtered solution in 20 mM Tris-HCl, 25 mM NaCl (pH 7.5)

**Molecular Weight** ~180 kDa daltons

**Purity** O-Glycanase is free of contaminating endo- and exoglycosidase activity. No protease activity was detectable after incubation of the enzyme with 0.2 mg resorufin-labeled casein for ~18 hours at 37°C according to the method described by Twining. The production host strain has been extensively tested and does not produce any detectable glycosidases.

**Activity** > 12 U/mg

**Optimum pH** Optimum: pH 5.0 Range: pH 5.0-6.0

**Specificity** O-Glycanase cleaves Gal  $\beta$  (1-3) GalNAc  $\alpha$ -as an intact disaccharide unit from serine or threonine residues of glycoproteins or glycopeptides. This disaccharide is the defining structural component for Core 1 type O-linked glycans. Cleavage of the glycosidic bond is between the GalNAc residue, in the alpha configuration, and the hydroxyl moiety of the amino acid side chain of the polypeptide. Substitutions of the disaccharide core with sialic acid, or a lactosaminic repeating unit of galactose-N-acetyl glucosamine or fucose, will block hydrolysis and prevent the liberation of the oligosaccharide from the protein. In order to expose the Core 1 type structure so that it is susceptible to the O-Glycanase action, extended oligosaccharides must first be treated with glycosidases, such as Sialidase A/NANase III, or in addition, treatment with a combination of  $\beta$  (1-4)-galactosidase and  $\beta$ -N-Acetylhexosaminidase/Hexase I. The enzyme has no activity on single  $\alpha$ -GlcNAc linked either to protein or carbohydrate. With the synthetic substrate analog, Gal  $\beta$  (1-3) GalNAc-p-nitrophenyl glycosidase, a  $K_m$  value of ~200  $\mu$ M was obtained. Interestingly, the enzyme is similar to other glycohydrolases and has been reported to have 'trans' glycosidase activity. Cleavage of the disaccharide unit is mediated by the formation of a covalent enzyme intermediate. The enzyme-bound glycan can be transferred to a number of hydroxylated acceptor molecules instead of displacement with water.

**Buffer** 5x Reaction Buffer 5.0 (250 mM sodium phosphate, pH 5.0)

### Storage and Shipping Information

**Storage** Shipped on cold pack for next day delivery. Store at 2-8°C. DO NOT FREEZE.