SIALIDASE A™

**SPECIFICATIONS**

<table>
<thead>
<tr>
<th>Product Code:</th>
<th>GK80040</th>
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<tbody>
<tr>
<td>Specific Activity:</td>
<td>40 U/mg</td>
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<tr>
<td>Activity:</td>
<td>≥5 U/ml</td>
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</tbody>
</table>

Shipped on ice pack for next day delivery.

**Storage:** 2-8°C  **DO NOT FREEZE**  
**Formulation:** A sterile-filtered solution in 20 mM Tris-HCl, 25 mM NaCl (pH 7.5)

Glyko® sequencing-grade Sialidase A™ (N-acetylneuraminate glycohydrolase, EC 3.2.1.18) cleaves all non-reducing terminal sialic acid residues from complex carbohydrates and glycoproteins (Figure 1). The relative cleavage rates for different linkages are:

\[ \alpha(2-6) > \alpha(2-3) > \alpha(2-8), \alpha(2-9) \]

In addition, Sialidase A will cleave branched sialic acids (linked to an internal residue). This property makes it unique among sialidases. High concentrations of enzymes and prolonged incubation times may be required for cleaving branched residues. To cleave only non-reducing terminal \( \alpha(2-3) \) unbranched sialic acid residues, use Glyko Sialidase S™.

Glyko Sialidase A is isolated from a strain of *E. coli* expressing a cloned gene from *Arthrobacter ureafaciens*. The enzyme has been extensively characterized using oligosaccharide standards.

Sialidase A, because of its purity and broad linkage specificity, has been extensively used in the analysis of both glycoproteins and glycolipids (Uchida *et al.*, 1977; Parekh *et al.*, 1985). It can be used in conjunction with the GKK-407 Signal™ DMB Sialic Acid Labeling Kit and GKI-4727 GlycoSep™ R HPLC Column (available from ProZyme) for the release and chromatographic analysis of sialic acid species.

Glyko Sialidase A is useful for:

- Structural analysis of oligosaccharides
- Determining sialic acid linkage (in conjunction with other sialidases having different specificities, such as Glyko GK80030 Sialidase C™ and GK80021 Sialidase S; these and other Glyko sialidases are available from ProZyme)
- Glycoprotein deglycosylation
- Removing heterogeneity from glycoproteins

**PRODUCT DESCRIPTION**

**Supplied Reagents** (research pack only)

5x Reaction Buffer  
(250 mM sodium phosphate, pH 6.0)
**Molecular Weight:** ~88 kD

**Purity:** The absence of exoglycosidase contaminants was confirmed by extended incubations with the corresponding pNP-glycosides. See certificate of analysis for specific assays performed.

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 (1984).

**Specificity:** Sialidase A cleaves all non-reducing terminal branched and unbranched sialic acids (see Figure 1). The enzyme releases $\alpha(2-3)$-, $\alpha(2-6)$-, $\alpha(2-8)$- and $\alpha(2-9)$-linked N-acetylneuraminic acid from complex carbohydrates. The initial rate of hydrolysis of $\alpha(2-6)$ linkages is reported to be approximately twice that of $\alpha(2-3)$-linked sialic acid however, in practice, this kinetic selectivity is of little consequence during extended incubations (Uchida et al., 1979). Effective digestion of glycolipid substrates is facilitated by the addition of a detergent, such as sodium taurodeoxycholate to the incubation (Saito et al., 1979).

**pH Range:**

- Optimum: pH 6.0
- Range: pH 4.5–8.0

50 mM sodium phosphate (pH 6.0) provides the optimal buffer for enzyme activity with sialyllactose, a standard substrate. With NAN-lactose the optimum pH is 5.0–5.5; with colominic acid (poly NeuAc) the optimum pH is 4.3–4.5. If glycosidase treatment is performed at a suboptimal pH because of glycoprotein solubility or activity requirements, expect some diminution in enzyme activity.

**ASSAY**

One unit of Glyko Sialidase A is defined as the amount of enzyme required to catalyze the release of 1 μmole of p-nitrophenol from pNP-α-D-N-acetylneuraminic per minute at pH 5.5 and 37°C.

**Additional Reagents (not supplied)**

- 250 μM 2-O-(p-nitrophenyl)-α-D-N-acetylneuraminic acid (Toronto Research Chemicals #N502501) in 100 mM sodium phosphate (pH 5.5)
- 0.5 M sodium carbonate

**Procedure**

Adjust spectrophotometer to read 405 nm.

Add 395 μl of substrate solution to two tubes and warm to 37°C.

Add 5 μl of enzyme to one tube and mix.

After 30 seconds, add 0.6 ml 0.5 M sodium carbonate to both tubes.

Blank spectrophotometer to control tube (without enzyme).

Read the absorbance at 405 nm.
**SUGGESTIONS FOR USE**

**Procedure for De-sialylation**

1. Add up to 100 μg of glycoprotein or 1 nmole of oligosaccharide to a tube.

   *Note: To cleave more than one nmole of substrate, increase reaction volume and enzyme proportionally.*

2. Add de-ionized water to a total of 14 μl.

3. Add 4 μl 5x Reaction Buffer.

4. Add 2 μl Sialidase A.

5. Incubate at 37°C for 1 hour.

   *NOTE: longer incubation times are necessary if branched sialic acids are present.*

De-sialylation may be monitored by SDS-PAGE if the size differential between native and de-sialylated protein is sufficient for detection.

**REFERENCES**


Figure 1 - Linkage specificities showing cleavable residues (in bold) for Sialidase A
Gal - galactose; Glc - glucose; Man - mannose; GalNAc - N-acetylgalactosamine; GlcNAc - N-acetylglucosamine; NeuAc - N-acetylneuraminic acid (sialic acid)

Gal β(1–3) GalNAc β(1–4) Gal β(1–4) Glc

GalNAc β(1–4) Gal β(1–4) Glc

NeuAc α(2–8) NeuAc α2

NeuAc α2

NeuAc α(2–3) Gal β(1–3) GlcNAc β(1–3) Gal β(1–4) Glc

NeuAc α(2–3) Gal β(1–4) GlcNAc β(1–2) Man α(1–6) Man β(1–4) GlcNAc β(1–4) GlcNAc

NeuAc α(2–6) Gal β(1–4) GlcNAc β(1–2) Man α(1–3)

NeuAc α(2–3) Gal β(1–4) GlcNAc β(1–4)

[NeuAc α(2–8)]" [NeuAc α(2–8) NeuAc α(2–9)]"