

## Product description

### for GlycoCleave® Neuraminidase Kit

#### Product description:

GlycoCleave kits combine the immobilized neuraminidase with reaction cartridges and ready-to-use buffer. The product is a suspension of immobilized neuraminidase (sialidase, N-acetylneuraminyl hydrolase, E.C. 3.2.1.18, from *Vibrio cholerae* culture filtrates). The enzyme particles (polymer particles, 65 µm) are stored in sodium acetate buffer pH 5.5 containing calcium chloride and sodium chloride. The immobilized neuraminidase has an activity of 1 U/mL settled particles.

#### Enzyme-Ligand:

*Vibrio cholerae* neuraminidase (E.C. 3.2.1.18) hydrolyses o-ketoside  $\alpha$ 2-3,  $\alpha$ 2-4,  $\alpha$ 2-6 and  $\alpha$ 2-8 bonds of terminal N-acetylneuraminic acid in oligosaccharides, glycoproteins, gangliosides and polysaccharides. This enzyme can be used for various investigations as for example structural characterization of glycoproteins, as a receptor-destroying enzyme in virology or to characterize and design the binding behavior of glycoconjugates. *Vibrio cholerae* neuraminidase requires  $\text{Ca}^{2+}$  ions for activity and is inhibited by the presence of EDTA. *Vibrio cholerae* neuraminidase preferentially hydrolyzes  $\alpha$ (2,3)-linkages of sialic acid, but will also cleave  $\alpha$ (2,6) and  $\alpha$ (2,8)-linkages. This preference for  $\alpha$ (2,3)-linkages is estimated at 260-fold. 1 U (unit) liberates 1 µmol N-acetylneuraminic acid from human acid  $\alpha$ <sub>1</sub>-glycoprotein incubated for 1 min at +37 °C and pH 5.5 in sodium acetate buffer (0.05 mol/L) containing 1 mmol/L calcium chloride.

The enzymatic activity of GlycoCleave® Neuraminidase EnzymeBeads is verified by the desialylation of bovine fetuin in comparison to asialofetuin (acid hydrolysis) carried out at 37 °C for 16 hours.

#### Kit Contents (for at least 3 enzyme reactions):

- 600 µL GlycoCleave immobilized neuraminidase (1 U/mL) settled particles as a 50 % slurry
- 2 x 30 mL sodium acetate buffer, pH 5,5; contains calcium, but no preservatives
- 3 x spin columns for separation of the desialylated protein from the immobilized enzyme, collection tubes, syringe adapters, 3 luer syringes

#### Storage:

GlycoCleave adsorbents should be stored at 2-8 °C. Buffer solution should be stored at 2-8°C and is then stable for 4 weeks.

#### Precautions / Disclaimer

This product is for research, laboratory and in vitro use only; not for drug or diagnostic use, food or food additives, household or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

For orders and technical support please contact:

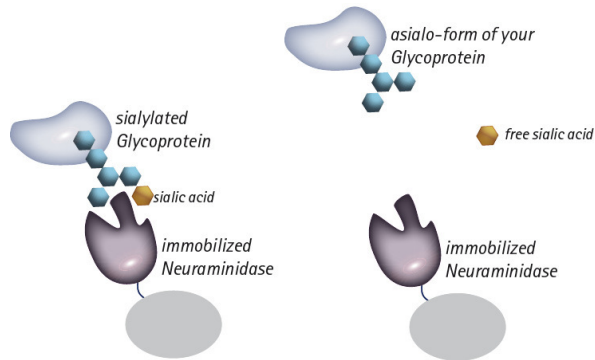
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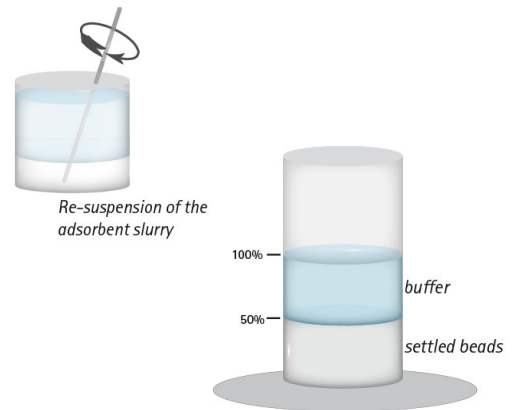
## Protocol for Using GlycoCleave® Neuraminidase EnzymeBeads

### Enzyme reaction:



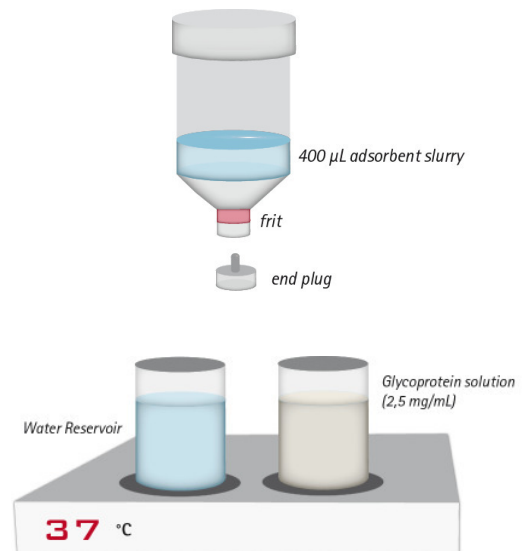
### Slurry preparation:

1. Re-suspend the provided adsorbent slurry in the bottle.
2. Transfer the homogeneous slurry to a graduated vessel. Allow the slurry to settle for at least one hour. Determine the settled adsorbent volume and adjust the slurry concentration to 50 %.

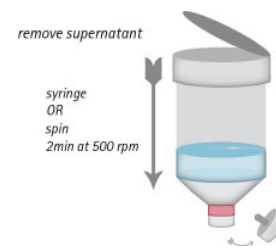


### Preparation:

3. Re-suspend the prepared adsorbent slurry and transfer 400  $\mu$ L of *Vibrio cholerae* neuraminidase particles to a fritted spin-column (200  $\mu$ L neuraminidase particles in each spin-column). Wash the adsorbent 2 times with 400  $\mu$ L reaction buffer. Seal the spin-column with the provided end plug.
4. Add 1,0 mL distilled water to a reaction tube and place the spin-column into this tube.
5. Dissolve up to 2,5 mg of your glycoprotein sample in 1mL reaction buffer in a sample vial. Preheat everything at 37°C in a thermo block.

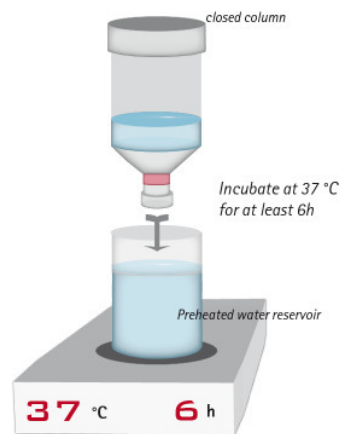


- Remove supernatant from the equilibrated enzyme fixed bed and add 400µL sample solution. Then close the spin-column with the screw cap and the provided end plug. Gently mix the particles and sample solution by shaking (avoid continuous shaking!).



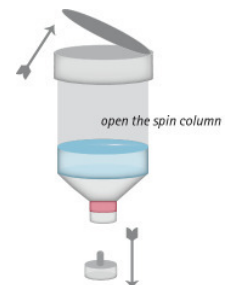
**Incubation:**

- Incubate the assembly at 37°C for at least 6 hours. The closed spin-column should be shaken temporarily.



**Separation of desialylated product from enzyme particles:**

- After 6 h incubation remove the plugs and place the spin-column into a collection tube. Then spin the column for 2 min at 500 rpm to pass the solvent through and separate your glycoprotein from the GlycoCleave particles. Wash the spin-column 4 times with 400 µL reaction buffer using the same method. Alternatively you can use the provided luer syringes and adapters to pass the buffer through the column.



**Sample collection and desalting:**

- Collect the whole flow-through in a tube (approx. 2 mL). For further applications, use appropriate methods.

