

Preciex® Stage-tip Instructions for:

Sialylated O-glycans enrichment

APPLICATIONS:

Sialylated O-glycans are glycans where sialic acid (Neu5Ac/Neu5Gc) caps O-linked glycans on serine/threonine residues of proteins. Sialic acids are negatively charged monosaccharides present as terminal epitopes, their abundance and types influence the clinical performance of therapeutic glycoproteins. For example, controlling immune recognition, playing strong role in cancer progression, regulating protein half-life and circulation, driving inflammation and infecting biology. Here we researched and developed an approach that is fast and reliable to analyse Sialylated O-glycans, which is the most labile glycan class due to its sensitivity of pH, alkaline β -elimination, sample handling and mass spectrometry ionization. An in-depth understanding structure-function relationship of these glycoproteins will speed up learning about the immune system, developing therapeutic biological drugs and vaccines.

O-glycan Release

Use commercial reagent (P/N:2605130) to release all O-glycans from raw protein, trapping reactive reducing end at the same time.

- a) Transfer desalted glycoproteins into 1.5 mL Eppendorf tube.
 - ✧ Recommended concentration: 1 – 10 mg/mL
 - ✧ Sample volume: less than 10 μ L of aqueous solution
- b) Add 20 μ L trapping-assisted release reagent
- c) Mix well by a Vortex mixer followed by a brief spin down, incubate the tube for 75 min at 37°C leaving the cap open.
- d) After the incubation, add immediately 70 μ L solution (i) to the tube.

Suitable solutions are:

- i. Conditioning and Equilibrating Buffer: 0.1% Acetic acid in Water
- ii. Loading Buffer: 0.1% Acetic acid in Water
- iii. Wash Buffer: as loading buffer
- iv. Elution Buffer: Water
- v. Conditioning Buffer: Water
- vi. Equilibrating Buffer: Water:Acetonitrile/10:90
- vii. Wash Buffer: Water:Acetonitrile/10:90
- viii. Elution Buffer: Water

We do not recommend any particular buffer, because each application has its own buffer and solutions. This products is in the method development stage and any application developed by you may be helpful.

Sialylated O-glycan enrichment

Conditioning and Equilibrating enrichment Stage-tip

Place a new Stage-tip and adaptor (P/N: 2110324) into a 2 mL microcentrifuge tube.

Pipetting 50 μ L solutions (i) as above. Centrifuge 1,500 g for 60 seconds, and repeat once.

Sample Loading

Transfer the equilibrated Stage-tip and adaptor into a new 2 mL microcentrifuge tube.

Load 20 μ L sample solutions (ii) as above. Centrifuge 1,500 g for 60 seconds. Typical loading capacity (P/N: 2111103) is 10 μ g of O-glycans. The elute can be reloaded on the tip if there is concern about the thoroughness of binding.

Sample Washing

Place Stage-tip and adaptor on a new collection tube.

Wash the packed bed 2x with 20 μ L volumes of the wash buffer(v) in order to wash out any other type of O-glycans and salts, centrifuge 2,000 g for 60 seconds.

Sample Release

Elute Sialylated O-glycans from the packed bed with 20 μ L of Elution buffer(vi) to a new collection tube. In order to elute all of the adsorbed bound molecule, repeat to do elution with 20 μ L of Elution buffer(vii), centrifuge 2,000 g for 60 seconds in each sample release step. Combine two elution together, Lyophilized for labeling.

Sialylated O-glycan labeling

Prepare Procainamide labeling solution as follows:

- Dissolve procainamide hydrochloride in a solution of DMSO and glacial acetic acid (e.g., 70:30 v/v) to a final concentration of approximately 40-50 mg/mL.
- Dissolve sodium cyanoborohydride or 2-picoline borane in the same DMSO/acetic acid solvent to a final concentration of approximately 60 mg/mL. Caution: Sodium cyanoborohydride is toxic. Handle with care in a fume hood.

Labeling Reaction:

- Dissolve the dried, released Sialylated O-glycans in 10 μ L of the labeling reagent. Add 10 μ L of the reducing agent solution. Vortex briefly to mix.
- Incubate the tube on a heat block at 50°C for 2.5 hr to perform a labeling reaction, leaving the cap open. After the labeling reaction, add immediately 100 μ L of ACN to the tube and mix well by pipetting up and down.

Labeled Sialylated O-glycan Clean-up

During the labeling reaction, Conditioning and Equilibrating a Glycan Stage-tip for sample clean-up.

Place a new Glycan Stage-tip and adaptor (P/N: 2110324) into a 2 mL microcentrifuge tube.

Pipetting 50 μ L solutions (v) as above. Centrifuge 1,500 g for 60 seconds, and repeat once.

Pipetting 50 μ L solutions (vi) as above. Centrifuge 1,500 g for 60 seconds.

Sample Loading

Transfer the equilibrated Stage-tip and adaptor into a new 2 mL microcentrifuge tube.

Load all labeling reaction solution into Glycan Stage-tip. Centrifuge 1,500 g for 120 seconds.

Sample Washing

Place Stage-tip and adaptor on a new collection tube.

Wash the packed bed 2x with 20 μ L volumes of the wash buffer(vii) in order to Remove labeling reagent and salts, centrifuge 1,500 g for 60 seconds.

Sample Release

Elute Sialylated O-glycans from the packed bed with 20 μ L of Elution buffer(viii) to a new collection tube. In order to elute all of the adsorbed bound molecule, repeat to do elution with 20 μ L of Elution buffer(viii), centrifuge 1,500 g for 60 seconds in each sample release step. Combine two elution together, dry them in a vacuum centrifuge and reconstitute (e.g., 25% acetonitrile in water) for MS analysis.

Please be sure of that Stage-tip is not dried out in all steps.

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