GLYCOWORKS *Rapi*Fluor-MS QUICK START PROTOCOL

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STREAMLINED PROTOCOL – 24 SAMPLE (3 x 8 FORMAT)

- Set heat blocks to at least 90 °C and 50 °C.
- Protocol is based on 1.5 mg/mL glycoprotein starting concentration.

STEP 1: Rapid Deglycosylation

1. Reconstitute 1 vial of the Intact mAb Mass Check Standard (1 mg/vial) in 670 µL 18.2 MΩ water to create a 1.5 mg/mL solution.

   Note: For glycoproteins with a formulation buffer containing nucleophiles or anionic reagents (e.g., His, Gly, Tris, PO_4^{3-}), if a Glycan C_{18} AX Column is applied for LC separation, it is highly recommended to desalt the sample with water prior to Step 1.
2. Prepare 3% (w/v) *Rapi*Gest™ SF by dissolving 3 mg of *Rapi*Gest SF Surfactant in 60 µL of Rapid Buffer and 40 µL of water, vortex.
3. Dilute PNGase F enzyme (35 µL) with 220 µL water for a total of 255 µL.
4. Add 10 µL of 1.5 mg/mL glycoprotein into the provided tube.
5. Add 10 µL of buffered 3% (w/v) *Rapi*Gest SF solution to above tube, aspirate to mix.
6. Heat at least to 90 °C for 3 minutes.
7. Cool at room temperature for 3 minutes.
8. Add 10 µL Rapid PNGase F and aspirate to mix.
9. Incubate at 50 °C for 5 minutes.
10. Cool at room temperature for 3 minutes.

STEP 2: Rapid Labeling of Glycosylamines

1. Add 110 µL of anhydrous DMF directly to one vial of 9 mg of *Rapi*Fluor-MS™ Reagent. Mix to solubilize.
2. Add 10 µL of the *Rapi*Fluor-MS solution to the deglycosylation mixture and aspirate to mix.
3. Allow the labeling to proceed at room temperature for 5 minutes.
4. Dilute the reaction with 360 µL of acetonitrile (ACN) and aspirate to mix.

STEP 3: HILIC Cleanup of Labeled Glycosylamines

1. Set up a GlycoWorks™ HILIC µElution Plate and add in shims or spacer and waste tray.
2. Condition wells by adding 200 µL of water per well.
3. Equilibrate wells by adding 200 µL 85% ACN.
4. Load ACN-diluted samples (~400 µL).
5. Wash wells with two (2) 600 µL volumes of 1% formic acid, 90% ACN.
6. Replace waste tray with sample collection tray loaded with 600 µL tubes.
7. Elute glycans with three (3) 30 µL volumes of SPE Elution Buffer into 600 µL tapered bottom inserts.
8. Dilute SPE eluate with 310 µL of the GlycoWorks SPE Diluent (DMF/ACN). Aspirate to mix.

   Note: For a Glycan C_{18} AX separation sample, either skip dilution Step 8 or dilute with 310 µL of water.
9. Cap the tubes with pre-slit cap mats.

► **For the complete Care and Use Manual, visit waters.com and search 715004903EN.**

► **For more details on this method, download Application Notes 720005506EN and 720007038EN.**