



NOTICE: ProZyme was purchased by Agilent in July 2018. Documents for products and product lots manufactured before August 2019 will contain references to ProZyme. For more information about these products and support, go to: www.agilent.com/en/contact-us.



GlykoPrep[®] Reduction-Denaturation Procedure

Note: this procedure is for use with GlykoPrep products only, in which glycoprotein samples are immobilized on an RX cartridge for N-Glycanase[®] digestion. It is not for use with in-solution N-Glycanase digestion protocols.

Not all glycoproteins are alike. For most, standard denaturation as described in the GlykoPrep Digestion Module protocol is sufficient to allow N-Glycanase full access to N-glycan cleavage sites; for others, more aggressive treatment may be required to achieve full denaturation. Additional denaturation *via* reduction may improve results in some cases.

This protocol presents two alternatives. The first, recommended procedure is Reduction-Denaturation: the sample is incubated with a Reductant, Tris(2-carboxyethyl)phosphine (TCEP) and Denaturation Reagent at high temperature (100°C) for 10 minutes prior loading onto the RX Cartridge. In the second, optional alternative, the sample is first incubated with the Reductant Tris(2-carboxyethyl)phosphine (TCEP) and Denaturation Reagent at high temperature (100°C) for 10 minutes and then alkylated with Iodoacetamide for 30 minutes in the dark prior loading onto the RX Cartridge.

For additional denaturation suggestions, consult the GlykoPrep Guidebook under Digestion Modules:

<http://www.prozyme.com/documents/TNGP100.pdf>

Required Reagents & Labware

TCEP, 500 mM (Thermo Scientific product number 77720 or equivalent)
Denaturation Reagent (supplied with the Digestion Module)
Iodoacetamide, 600 mM in water, made same day - *optional, for Reduction -Alkylation-Denaturation Procedure*
96-well PCR plate (96 Eppendorf 30129300, PCR, Full Skirt, PolyPro)

Additional Equipment

PCR thermocycler with heated lid

Sample Quantity

It is recommended to bring the protein samples to 1 mg/ml prior to this procedure.
Example: sample concentration 1 mg/ml: use 55 μ l of sample.

Procedure

NOTE: Perform procedure A or B.

A. Reduction-Denaturation (recommended)

Set PCR thermocycler to 100°C.

Using a multichannel pipette, add 10 μ l of 500 mM TCEP to each 55 μ l protein sample in a 96-well PCR plate (96 Eppendorf 30129300, PCR, Full Skirt, PolyPro).

Add 45 μ l of Denaturation Reagent to each sample and mix several times by pipette action.
Seal the plate with foil.

Heat in PCR thermocycler for 10 minutes at 100°C.

Spin the plate at 1000 x g for 1 minute.

Allow plate to cool at room temperature for 10 minutes.

Remove the foil and proceed to the GlykoPrep RX loading step or the GlykoPrep-*plus* Immobilization and Digestion Protocol, following instructions page 3.

B. Reduction-Alkylation-Denaturation (optional)

Set PCR thermocycler to 100°C.

Using a multichannel pipette, add 10 μ l of 500 mM TCEP to each 55 μ l protein sample in a 96-well PCR plate (96 Eppendorf 30129300, PCR, Full Skirt, PolyPro).

Add 35 μ l of Denaturation Reagent to each sample and mix several times by pipette action.
Seal the plate with foil.

Heat in PCR thermocycler for 10 minutes at 100°C.

Spin the plate at 1000 x g for 1 minute.

Allow plate to cool at room temperature for 10 minutes.

Remove the foil.

Add 10 µl of 600 mM Iodoacetamide to each sample, and mix well by pipette action. Cover and incubate in the dark at room temperature for 30 minutes.

Proceed to the GlykoPrep RX loading step or the GlykoPrep-*plus* Immobilization and Digestion Protocol, following instructions page 3.

If proceeding to GlykoPrep (manual, spin), add 100 µl of denatured sample to the RX Cartridge.

If proceeding to GlykoPrep-*plus* on the Agilent Bravo, make the following modifications in the Reagent Volume Calculator and Immobilization and Digestion Protocol settings:

1. Reagent Volume Calculator Settings (See Figure 1):

- a. Set Sample Concentration to 0.5 mg/ml
- b. Set Denaturation Reagent Factor to 0

| Calculator Data Entry | Recommended | Actual |
|--|-------------|--------|
| Number of Samples | 8–96 | 96 |
| Number of plate columns used | 12 | 12 |
| Sample Preparation | | |
| Target Load (µg) | 5–50 | 50 |
| Sample Concentration (mg/mL) | 0.05–5 | 0.5 |
| Required Glycoprotein Starting Sample Vol (µL) | | 110 |
| Denaturation Reagent Factor (x:1) | 1 | 0 |
| Denaturation Reagent Volume (µL) | | 0 |
| Denatured Sample Load Volume (µL) | | 100 |

Figure 1. Alternate Reagent Volume Calculator settings.

2. Immobilization & Digestion Protocol Application Settings (see Figure 2):

- a. Set Denaturant Volume to 0 µl
- b. Set Starting Sample Volume to 110 µl

The screenshot displays the '3 Immobilization & Digestion Protocol' application settings. The 'Application Settings' table is as follows:

| Parameter | Value | Units |
|-------------------------------------|-------|---------|
| Denaturant Volume | 0 | µL |
| Starting Sample Volume | 110 | µL |
| Denatured Sample Load Volume | 100 | µL |
| Sample Loading Flow Rate | 5 | µL/min |
| Temperature Set Point for Digestion | 45 | °C |
| Duration of Digestion Step | 30 | minutes |

The status bar at the bottom indicates 'Status 3 Run Complete, Module Idle' and includes a 'Run Protocol 3' button.

Figure 2. Alternate Immobilization & Digestion Protocol Application Settings.

Supporting Data

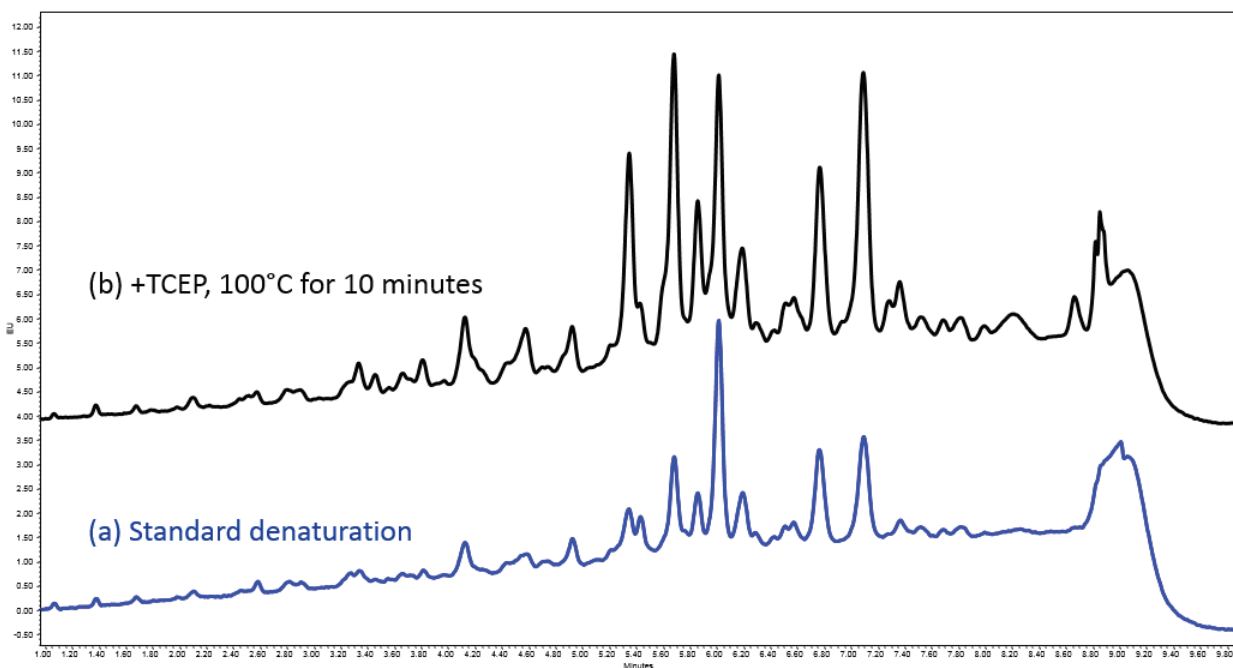


Figure 3. 2-AB labeled N-glycans from human follicle stimulating hormone (FSH). N-Glycans were prepared with GlykoPrep using either (a) standard GlykoPrep denaturation or (b) optional reduction-denaturation as described in protocol A on page 2.