ProteoExtract® Glycopeptide Enrichment Kit

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About the Kits

ProteoExtract® Glycopeptide Enrichment Kit 1 kit 72103-3

Description

The development of methods to analyze glycosylation of proteins has historically been largely neglected. This has changed recently due to findings that glycosylation influences crucial biological and pharmaceutically relevant signaling pathways, as well as recent developments in mass spectrometry (MS) that make in depth glycosylation analysis possible. Due to the low abundance of glycopeptides in complex mixtures, glycoanalysis studies require highly selective and sensitive methods for sample preparation. For pharmaceutical industry purposes, where protein glycosylation is monitored as a matter of routine, efficient and reproducible methods are especially important.

The ProteoExtract® Glycopeptide Enrichment Kit uses a proprietary ZIC® Glycocapture Resin in combination with unique buffers to selectively enrich for glycosylated peptides (high-mannose, hybrid and complex type) in the presence of abundant non-glycosylated peptides without preference for particular glycan structures or loss of distinct glycans. The protocol is optimized to efficiently remove non-glycosylated peptides with almost complete glycopeptide recovery (quantitatively determined by N-Glycan mapping), allowing subsequent site-specific determination of the glycan structure and parallel analysis of the attached peptides via MS. The enriched glycopeptide fraction is directly compatible with downstream analysis by MALDI and ESI mass spectrometry.

Components

- 2.7 ml ZIC® Glycocapture Resin suspension
- 0.3 ml ZIC® Binding Buffer
- 7.5 ml ZIC® Wash Buffer
- 5.5 ml ZIC® Elution Buffer

Storage

Store all components of the kit at 4°C for up to 6 months.

Equipment and materials required but not supplied

- Micropipettes and tips; 10 µl, 200 µl , and 1 ml
- Microcentrifuge and rotor for >10,000 x *g*
- 1.5 ml microcentrifuge tubes
- Thermomixer
- Evaporator (e.g., SpeedVac®)
- High-grade acetonitrile

ProteoExtract® Glycopeptide Enrichment Kit Protocol

Considerations Before You Begin

Table 1. Preparation Instructions for Reagents.

Material provided	Preparation Instructions
ZIC® Glycocapture Resin	Ready to use
ZIC® Binding Buffer	Add 2.7 ml high-grade acetonitrile*
ZIC® Wash Buffer	Add 18 ml high-grade acetonitrile*
ZIC® Elution Buffer	Ready to use

*Use only high-grade acetonitrile for use in preparing Glycopeptide Enrichment Kit reagents.

The following protocol is designed for glycopeptide enrichment from a 10 µl sample with a total protein concentration of 2–4 mg/ml. This protocol can be scaled up or down depending on experimental requirements. Use sample:ZIC® Binding Buffer:ZIC® Glycocapture Resin:ZIC® Wash Buffer:ZIC® Elution Buffer at the ratio of 1:5:5:15:7.5–10. e.g., for a 10 µl sample, use 50 µl ZIC® Binding Buffer, 50 µl ZIC® Glycocapture Resin, 150 µl ZIC® Wash Buffer, and 75–100 µl ZIC® Elution Buffer.

All incubations should be performed at room temperature.

Sample Preparation

Samples should be digested (e.g., with trypsin) prior to use with this kit. If the salt concentration in the sample exceeds 150 mM or the urea concentration exceeds 2 M, exchange buffer or dilute with ultra pure water and appropriately adjust reagent volumes. The pH of the sample should be between 2 and 8.

Protocol for Maximum Yield

1. Dilute 10 µl digested sample by adding 50 µl ZIC® Binding Buffer.
2. Mix ZIC® Glycocapture Resin by vortexing until completely resuspended.
3. Transfer 50 µl of the homogenous suspension to a new microcentrifuge tube.
4. Centrifuge the tube for 1–2 min at 2000–2500 x *g*. Remove the supernatant completely and discard.
5. Add the diluted sample to the ZIC® Glycocapture Resin.
6. Mix by pipetting up and down, and incubate with agitation for 10–20 min. The use of a thermomixer at 1200 rpm is recommended for agitation. Alternatively, vortex the tube briefly several times during the incubation.
7. Centrifuge the tube for 1–2 min at 2000–2500 x *g*. Remove the supernatant completely and discard.
8. Add 150 µl ZIC® Wash Buffer to the ZIC® Glycocapture Resin.
9. Mix by pipetting up and down, and incubate with agitation for 5–10 min. The use of a thermomixer at 1200 rpm is recommended for agitation. Alternatively, vortex the tube briefly several times during the incubation. Centrifuge the tube for 1-2 min at 2000-2500 x g. Remove the supernatant completely and discard.
10. Repeat steps 8 and 9 two times for a total of three washes.
11. Elute glycopeptides by adding 75–100 µl ZIC® Elution Buffer.
12. Mix by pipetting up and down, and incubate with agitation for 2–5 min. The use of a thermomixer at 1200 rpm is recommended for agitation. Alternatively, vortex the tube briefly several times during the incubation.
13. Centrifuge the tube for 1–2 min at 2000–2500 x *g*.
14. Transfer the supernatant to a new microcentrifuge tube.
15. Centrifuge supernatant for 2 min at 10,000 x *g*.
16. Transfer the supernatant to a new microcentrifuge tube avoiding the transfer of resin particles. This contains the eluted glycopeptides.
17. Completely evaporate eluate using evaporator and resuspend in a suitable buffer for downstream analysis.

Alternative Elution Procedure for Direct Processing

1. Perform steps 1–10 as described above for “Protocol for Maximum Yield.”
2. After the last wash step, completely discard the supernatant.
3. Dry the ZIC® Glycocapture Resin for 5–10 min using evaporator until it appears white. Do not over dry as this will reduce yield. Alternatively, the ZIC® Glycocapture Resin can be dried by incubating at 40–60ºC for several minutes until it starts to appear white
4. Elute glycopeptides by adding 40 µl of a suitable buffer for downstream analysis (e.g., 50 mM NH_4HCO_3, pH 8 for PNGaseF digest).
5. Mix by pipetting up and down, and incubate with agitation for 10 min. The use of a thermomixer at 1200 rpm is recommended for agitation. Alternatively, vortex the tube briefly several times during the incubation.
6. Centrifuge the tube for 1–2 min at 2000–2500 x *g*.
7. Transfer the supernatant to a new microcentrifuge tube.
8. Centrifuge supernatant for 2 min at 10,000 x *g*.
9. Transfer the supernatant to a new microcentrifuge tube avoiding the transfer of resin particles. This contains the eluted glycopeptides.
10. Eluted glycopeptides can be used directly for further analysis.

Appendix

Assay Characteristics and Examples

The data in Figure 1 demonstrate that the ProteoExtract® Glycopeptide Enrichment Kit results in efficient and specific glycopeptide capture. The predominant signals are derived from glycopeptide ions (marked with arrows), which were digested with PNGaseF after enrichment in order to obtain one significant signal. The majority of non-glycosylated peptides are completely removed (low background). The data in Figure 2 show the compatibility with online LC-ESI MS/MS and offline MALDI MS and the site-specific determination of glycan structure as well as peptide backbone.

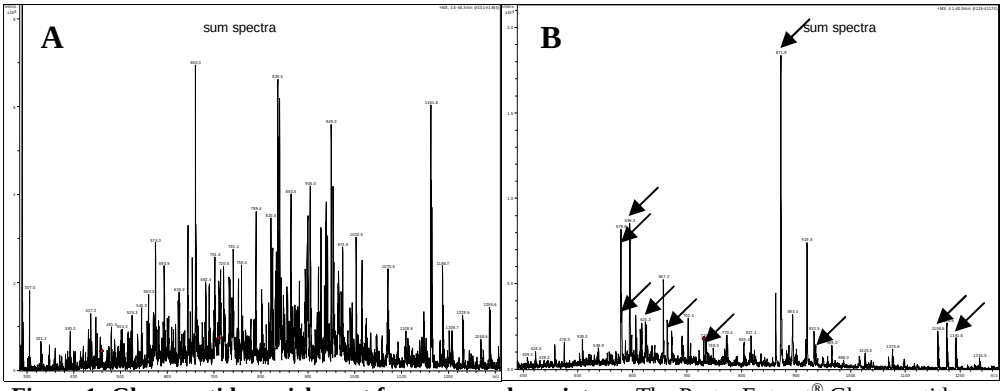

Figure 1: Glycopeptide enrichment from a complex mixture. The ProteoExtract® Glycopeptide enrichment kit allows for selective and sensitive glycopeptide enrichment with low background from complex mixtures. A complex peptide mixture derived from a tryptic digest of bovine fetuin, α1 acid glycoprotein, RNaseB, histone, BSA and human IgG was processed using the ProteoExtract® Glycopeptide Enrichment Kit as outlined in the detailed user protocol. Mass spectrometry analysis was performed using ESI-LC/MS equipment operated in positive mode. (**A**) Sum spectra of unprocessed sample (**B**) Sum spectra of PNGaseF digested glycopeptides recovered using the ProteoExtract® Glycopeptide Enrichment Kit.

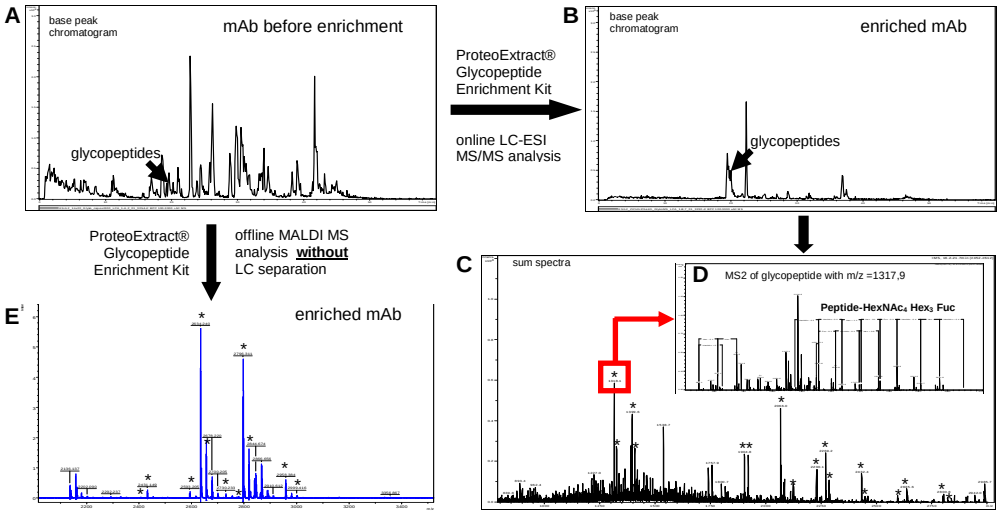

Figure 2: Glycan structure determination of an enriched monoclonal antibody by online LC-ESI MS/MS and offline MALDI MS. Enrichment of a monoclonal antibody using the ProteoExtract® Glycopeptide Enrichment Kit results in an enhanced glycopeptide recovery and glycan structure determination and enables direct offline MALDI MS analysis without LC separation. 125 pmol monoclonal antibody (mAb) was digested with trypsin and enriched using the ProteoExtract® Glycopeptide Enrichment Kit. For determination of attached glycan structure and heterogeneity, eluates were directly analyzed by online LC-ESI MS/MS (**A-D**) and offline MALDI-TOF MS (**E**). (**A**) Base peak chromatogram of unprocessed sample. (**B**) Base peak chromatogram of enriched sample. (**C**) Sum spectra of enriched sample. (**D**) MS2 spectra of the glycopeptide with m/z 1317.9. (**E**) Eluate was directly spotted on a target and analyzed by offline MALDI-TOF MS without LC separation.