protea®
Product Information Document

GPR LC-MS Top-Down Prep Kit, Low MW

Catalog Number: GPR-050

PRODUCT DESCRIPTION:

GPR Application Kits are used for post-GPR processing of recovered proteins from gel protein recovery experiments prior to analysis. All Application Kits contain a surfactant degradation reagent for breakdown of the Progenta Anionic Acid Labile Surfactant that is contained in the GPR Electroelution Buffer.

PROTOCOL:

A. Sample collection and detergent degradation:
1) Collect sample from protein GPRchip into microcentrifuge tube using a micropipette.
2) Add Surfactant Degradation Reagent (10X) to the sample to achieve a 10-fold dilution (e.g. add ~15 µL of Surfactant Degradation Reagent to a 150 µL GPR sample) and incubate at room temperature for 15 – 30 minutes (15 minutes for MALDI analysis ; 30 minutes for ESI analysis).

B. Desalt protein sample using a C_8 SpinTip:
1) Ensure that the packing material is at the bottom of the tip by gently tapping the tip to displace any packing material sticking to the top cap.
2) Place a centrifuge adaptor onto a 2 mL centrifuge tube.
3) Remove the cap from the SpinTip and place into the centrifuge adaptor.
4) Wash the SpinTip to wet the packing material by adding 50 µL of Equilibration Solution to the top of the SpinTip using a micropipette. Centrifuge the system at 4000 x g for 3 min. Repeat the SpinTip wash.
5) Rinse the SpinTip by adding 50 µL of Sample Reconstitution and Rinse Solution to the top of the SpinTip. Centrifuge the system at 4000 x g for 3 min. Repeat the SpinTip rinse.
6) Load 10 to 200 µL of the GPR Sample Solution by adding it to the top of the SpinTip and centrifuging the system at 4000 x g for 3 min. Additional sample volumes can be added in 200 µL aliquot cycles.
7) Wash the sample to elute salts and other non-retained components by adding 100 µL of the GPR Rinse Buffer for ESI to the top of the SpinTip. Centrifuge the system at 4000 x g for 3 min. Repeat the SpinTip sample wash.
8) Transfer the SpinTip and adapter to a new clean centrifuge tube to collect the sample during elution.
9) Elute the sample by adding 100 µL of Elution Solution to the top of the SpinTip. Centrifuge the system at 4000 x g for 3 min.
1. NOTE: Monitor the level of liquid in the waste centrifuge tube being careful the flow through volume does not cover the bottom point of the SpinTip.
10) Dry down sample in a lyophilizer or SpeedVac.
11) NOTE – for proteins > 75 kDa, a C_4 SpinTip should be used

C. LC-MS Analysis of Intact Proteins
1) Add 10-20 µL of the LC aqueous (sample loading) solution to the solid sample and sonicate briefly to dissolve the sample.
2) Load sample into sample loop on LC-MS system.
3) Load sample from sample loop onto C_8 column and begin LC-MS analysis.

PRODUCT CARE AND STORAGE:

The GPR LC-MS Top-Down Prep Kits should be stored at room temperature out of direct sunlight. The Surfactant Degradation Reagent contains trifluoroacetic acid (TFA) and should be used with the proper personal protective equipment. Each GPR LC-MS Top-Down Prep Kit has a shelf life of two years when properly stored.

ORDERING INFORMATION:

Catalog Number: GPR-050-1

Contains Surfactant Degradation Reagent (GPR-025-10mL) and C_8 GPR SpinTips Sample Prep Kit for ESI (SP-161-24)