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APPLICATION NOTE

Ultrasensitive proteomics for microdissected fresh frozen tissue samples using the Bioruptor® Pico for sample preparation

INTRODUCTION

Histology-defined proteomics investigations are a fundamental tool in modern molecular pathology. Molecular signatures of specific cells must be detected within their correct histopathological context to draw conclusions regarding disease development and progression. The proteomics group at the Fondazione Pisana per la Scienza ONLUS is developing advanced mass spectrometry protocols for proteomics characterization of oncological and neurological samples. This involves mass spectrometry imaging (MSI), for its seamless integration with histopathological analysis, as well as histology/MSI directed laser capture microdissection for in-depth quantitative proteomics [1,2]. The LC-MS/MS based proteomics on microdissected samples requires the miniaturization of the traditional bottom-up protocols.

MATERIAL REQUIRED

- Fresh frozen tissue sections of 10-15 µm of thickness mounted on polyethylenenapthalate (PEN) coated slides.
- Laser capture microdissection system
- Bioruptor® Pico
- Lysis buffer: 10 mM HEPES, 1% SDS, 5mM EDTA, 5mM EGTA, pH 8.5
- Micro BCA (Pierce®) Preparation Kit protocol.

PROCEDURE:

- Stain the tissue sections with haematoxylin, adding protease inhibitors to the water and 70% EtOH based washes.
- Isolate the areas of interest with laser capture microdissection collecting minimum 0.5 - 1 mm^2 in an adhesive cup tube.
- Carefully transfer the microdissected tissues from the adhesive cup in to a clean tube and add 20 µL of a solution of 50/50 lysis buffer and trifluoroethanol.

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- Sonicate the samples with the Bioruptor® Pico for 10 cycles (30 sec ON/ 30 sec OFF).
- Quantify the amount of extracted proteins with a modified microBCA test (Pierce®): prepare a working reagent solution (25:24:4 A:B:C), prepare BSA calibration standards 0-120 ng/µl, mix in proportion 2:1 working reagent/sample solution and incubate at 60 °C for 1 h; measure the absorbance at 562 nm.[2]
- Denature the extracted proteins by heating the tube to 95 °C for 5 minutes.
- Continue with reduction, alkylation and digestion using a specific miniaturized sample preparation protocol to achieve the highest number of identified proteins.[1,2]
- Desalt the samples before injection onto a NanoLC – MS/MS system.[1,2]

RESULTS

Typically, 1 mm^2 of brain cortex provides c.a. 0.8 µg of protein. However, the amount of extracted proteins per unit area is tissue-dependent and may vary even across the same histological area, Figure 1.A. An ultrasensitive proteomics protocol based on 10-plex TMT quantitation, MS3 and high pH fractionation enabled high precision in-depth quantitative analysis (Figure 1.B).

CONCLUSION

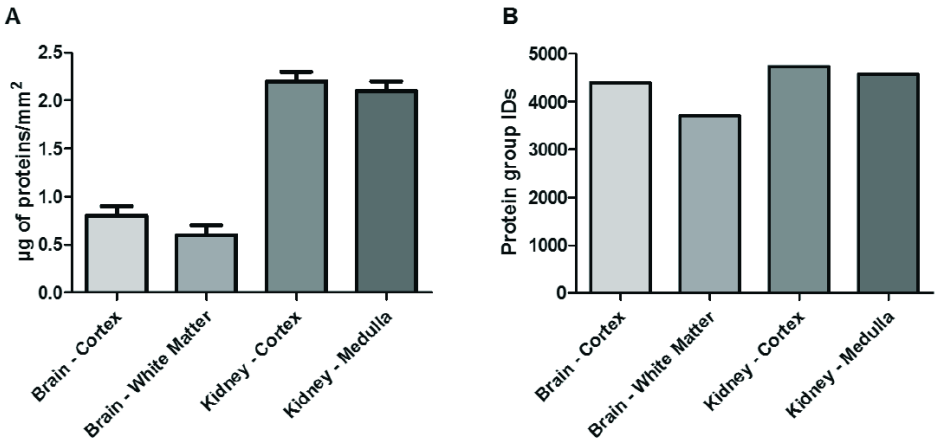

Figure 1.

A Protein yield for 1 mm^2 of tissue microdissected from different histological regions (10 µm cerebral tissue sections, 15 µm kidney tissue sections). [1,2,3]

B Protein groups identified for different histological regions, using an Easy-nLC 1000 coupled to an Orbitrap Fusion, following high pH fractionation of the complex peptide mixtures. [1, 2, 3] [Results adapted from [1, 2]]

This protocol, specifically designed for ultrasensitive proteomics, enables high throughput quantitative proteomics study on microdissected tissue samples. The Bioruptor® Pico provided high efficiency protein extraction, essential for the analysis of proteins from micro-scale tissue samples.

PUBLICATIONS

1. Dilillo M., Pellegrini D., Ait-Belkacem R., de Graaf E.L., Caleo M., McDonnell L.A. Mass Spectrometry Imaging, Laser Capture Microdissection, and LC-MS/MS of the same tissue section, Journal of Proteomics Research 16 (2017) 2993-3001. http://pubs.acs.org/doi/abs/10.1021/acs.jproteome.7b00284
2. de Graaf E.L., Pellegrini D., McDonnell L.A. Set of novel automated quantitative microproteomics protocols for small sample amounts and its application to kidney tissue substructures, Journal of Proteomics Research 15 (2016) 4722-4730. http://pubs.acs.org/doi/abs/10.1021/acs.jproteome.6b00889
3. Unpublished results