High Pressure-Assisted Extraction for the Improved Proteomic Analysis of FFPE Tissue

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OVERVIEW

• Formamide-induced crosslinks hampers the analysis of Formalin-fixed, paraffin-embedded (FFPE) tissue by MS
• When FFPE mouse liver was extracted using heat and elevated pressure (40,000 psi), there was a 4-fold increase in protein extraction efficiency and up to a 30-fold increase in the number of non-redundant proteins identified by mass spectrometry
• Heat augmented with high hydrostatic pressure (40,000 psi) improves the quality and yield of proteins extracted from archival tissue

METHODS

Preparation of FFPE Tissue: The liver from a female BALB/c mouse was given as a gift under the secondary use provision by the National Disease Research Interchange. The liver was excised under a sterile surgical hood and one half was immediately snap-frozen in Tissue-Tek O.C.T. compound (Sakura Finetek). The other half was fixed for 48 h at 4°C in 10% phosphate-buffered formalin (Thermo Fisher Scientific). The formalin fixed tissue was washed for 30 minutes with distilled water and then dehydrated through a series of graded alcohols and xylenes for 1 hour each (70%, 85%, 100%, and ethanol), and two changes of xylene. The tissue was incubated overnight at 85°C in ParaPlast Plus paraffin (Thermo Fisher Scientific) before embedding.

Deparaffinization and Recovery of Proteins: 20 micron sections of the FFPE mouse liver were deparaffinized by incubating the surrogate through two changes of xylene for 10 minutes each. The surrogates were rehydrated through a series of graded alcohols for 10 minutes each (100%, 85%, 70%, and ethanol). The deparaffinized surrogates were incubated in distilled water for a minimum of 30 minutes.

The rehydrated sections were reprocessed in 50 mM Tri-HCl pH 4.7, 7, 8, or 9 with 2% (v/v) SDS (EB1) or 100 mM Tri-HCl pH 8, 100 mM dibutylthritol (DTT), 4% SDS (EB2). The suspensions were homogenized by two 10 second cycles using a Fison Dismembrator (model 550). Next with a 0.125 inch tapered microtip (Fisher Scientific). The homogenized tissue surrogates or FFPE liver were incubated at 100°C for 30 minutes followed by 80°C for 2 hours under atmospheric pressure (14.7 psi) or 40,000-45,000 psi as previously described (2).

LC-MS of FFPE Mouse Liver: 40 μg of each fresh and FFPE liver extract was separated by SDS-PAGE on precast NuPAGE Bis-Tris 4-12% polyacrylamide gels using MES-SDS running buffer at pH 7.3 (Invitrogen). The gels were stained using SilverQuest silver staining (Invitrogen) Each gel lane was divided into approximately 10 bands per lane, and each band was digested overnight at 37°C using a standard in-gel trypsin digestion protocol.

The tryptic peptides were separated on an Agilent 1100 nanoflow LC system coupled on-line to a linear ion trap mass spectrometer (LTQ, ThermoElectron). The LTQ-MS was operated in a data-dependent mode where each full MS scan was followed by up to two internal mass scans. The proteins were then analyzed by MALDI-TOF-MS using a Voyager-DE STR Biospec. The MS/MS spectra were analyzed by SEQUEST (ThermoElectron). The data was analyzed against a combined Uniprot non-redundant mouse proteome database containing 35,795 protein sequences downloaded January 2010 (www.expasy.org). Only peptides with conventional trypsin termini (allowing for up to two internal missed cleavages), possessing delta-correlation scores <0.01 and charge state-dependent cross-correlation (Xcorr) criteria as follows were considered as peptide identifications: >1.9 for +1 charged peptide, >2.2 for +2 charged peptide, and >3.5 for +3 charged peptide. A reverse-database search was performed using the above database resulting in a calculated false-positive rate of ~2%.

RESULTS

Recovering Stored Pathology Specimens with High Pressure + Heat:

• Improves total protein recovery by 4-fold.
• Increases the number of unique proteins identified over samples recovered with heat alone.

• Most methods for FFPE tissue extract tryptic peptides only.

Extraction of whole proteins with elevated pressure allows for validation by ELISA, Western blot, etc.

Table 1. MS analysis for FFPE and matched fresh mouse liver

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Pressure (psi)</th>
<th>Extraction condition</th>
<th>% Protein Extraction *</th>
<th>Unique Protein IDs</th>
<th>Unique peptide IDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh, 0°C</td>
<td>14.7</td>
<td>100°C C + 80°C EB1</td>
<td>100%</td>
<td>238</td>
<td>0350</td>
</tr>
<tr>
<td>FFPE, 37°C</td>
<td>14.7</td>
<td>100°C C + 80°C EB1</td>
<td>100%</td>
<td>344</td>
<td>0439</td>
</tr>
<tr>
<td>FFPE, 40°C</td>
<td>40,000</td>
<td>100°C C + 80°C EB1</td>
<td>77%</td>
<td>626</td>
<td>5192</td>
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<tr>
<td>Fresh, 1°C</td>
<td>14.7</td>
<td>105°C C + 80°C EB1</td>
<td>100%</td>
<td>107</td>
<td>107</td>
</tr>
<tr>
<td>FFPE, 1°C</td>
<td>14.7</td>
<td>105°C C + 80°C EB1</td>
<td>100%</td>
<td>107</td>
<td>107</td>
</tr>
<tr>
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<td>105°C C + 80°C EB1</td>
<td>79%</td>
<td>107</td>
<td>107</td>
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</tbody>
</table>

* Proteins were quantified by the ratio of unique peptide IDs to total peptide IDs.

REFERENCES


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