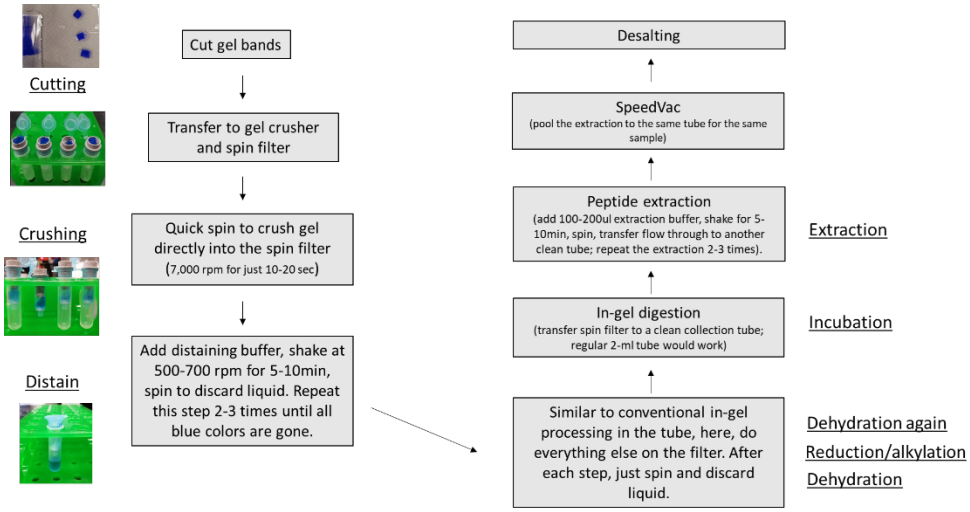
SOP

UNIVERSITY OF DELAWARE MASS SPECTROMETRY FACILITY

On-filter in-gel digestion for proteomics with E2technology

Summary

This SOP pertains to processing biological samples for high resolution mass spectrometry (MS) based proteomics analysis. The protocol describes protein digestion from SDS PAGE gel bands. Other procedures including cell lysis and peptide desalting are provided separately. Here is an illustrative workflow for **on-filter in-gel digestion** using the newly developed **E2technology** by UD MS Facility.



1. Materials and Equipment.

1) Gel crusher: please contact UD MS Facility.
2) E3filter: CDS Analytical, cat #: 70-2019-3101-0
3) TEAB: Triethylammonium Bicarbonate Buffer, 1M stock from Fisher (cat #: 60-044-973).
4) TCEP/CAA: 10mM/40mM in 50mM TEAB.
5) Destaining buffer: 50 mM acetonitrile in 50mM ammonium bicarbonate.
6) Extraction buffer: 50% ACN/50% water/0.1% formic acid.
7) Trypsin (Sequencing Grade Modified Trypsin, Fisher Catalog Number: PR-V5111. 20ug/vial. Add 2mL 50mM TEAB solution to each vial to make 10ng/ul Trypsin solution. Store in -80°).
8) Please refer to **Recommended consumables and supplies for proteomics experiments**.

2. Experimental Procedures.

1) **Gel cut**
   Cut gel bands of your interest with razor blade.
2) **Crushing gel**
   Assemble the gel crusher into the E3filter and collection tube, transfer gel bands to it. Spin at 7,000 for a few seconds.
3) **Destain**
   Remove and recycle gel crusher. Add 100-300uL destaining buffer, shake 5-10min, spin and discard solution; repeat 2-3 times until the blue color completely disappears.
4) **Dry/ Dehydrate**
   Add 100-200uL 100% ACN solution, shake 5-10 min, spin and discard solution; repeat 2-3 times until the gel pieces are firm/hard.
5) **Reduction/alkylation**

Add 10 mM TCEP and 40mM CAA to gels, incubate at 70ºC for 5 min. Spin and discard flow through.

6) **Dry/ Dehydrate**
   Repeat Step 4.
7) **Add enzyme**
   Add 10-50uL* Trypsin solution (10ng/uL) to just cover the gel pieces, and put the tubes into ice for 30 minutes.
   *This volume will vary from sample to sample depending on the size of gel pieces. Don't add too much trypsin solution. The volume just covering the gels is fine.
   *After 30 minutes, remove extra trypsin solution from the top of gels if necessary; or add a little bit more if the solution has been soaked up.
8) **Add another 20-50uL HPLC water to cover the trypsin solution; 37°C overnight with gentle shaking.**

(Next day)

9) **Peptide extraction**
   Add 100-200uL 50% ACN/0.1%TFA, vortex 10-30min, spin and collect the solution (into a new tube); Repeat the above steps THREE times;
10) **Dry the peptide solution and it is ready for desalting.** All peptide samples should be desalted before LCMS analysis.

Note: To minimize keratin contamination, *lab coat/gloves/mask* MUST be worn. The gel may be processed in a clean room or in the hood.