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UNIVERSITY OF SOUTH ALABAMA
MASS SPECTROMETRY
CORE FACILITY

IN-GEL PROTEIN DIGESTION FOR COOMASSIE BLUE STAINED GELS

** Proper sample preparation is crucial for successful MS results. Please consult the MS facility regarding sample preparation prior to bringing your samples for analysis. **

It is critical to store your prepared gel under water, in a clean covered container to minimize keratin contamination. Do not prepare samples too far in advance prior to MS analysis. To cut gel spots, place gel slice on a glass plate or cutting board. Place ~20µL of H_20 on the slice to prevent dust from hitting the gel slice while you are working. Through the water droplet, use a cutting device to cut the slice into smaller pieces (~1mm in size). Transfer gel spots into an Eppendorf tube. Take care to ensure the gel pieces do not get removed when aspirating solutions away from the gel pieces throughout this protocol.

Day 1

Prepare the following Solutions:

- 200mM ABC (ammonium bicarbonate): 0.78g of ABC in 50mL of H_20
- Neat ACN (Acetonitrile)
- 50mM ABC: 50ml of 200mM ABC in 150mL of H_20
- 50% 200mM ABC/ 50% methanol: 25ml of 200mM ABC in 25mL of MeOH
- Urea Solution (8M urea / 10mM TCEP / 5mM EDTA / 0.1M ABC): 0.481g urea, 500µL of 200mM ABC, 10µL of 0.5M EDTA, 20µL of 0.5M TCEP, 120µL of H_20
- Trypsin Solution (DO NOT MAKE UNTIL STEP 10): 2 µL of concentrated trypsin (less than one week old), 99µLof 200mM ABC, and 99µL of H_20. Trypsin stock: Add 100µl of trypsin suspension buffer to lyophilized trypsin (Promega V511a).
- IAA (500mM): 9.3mg Iodoacetamide in 100µL of H_20

Procedure:

1. Place the Coomassie Blue stained gel pieces ~ 1mm in size in a 1.5mL eppendorf tube.
2. Add 200µL of 50% 200mM ABC/ 50% methanol solution to gel pieces. Shake for 15 min. Remove solvent with a 200µL pipette using gel loading tips.
3. Repeat step 2 until gel pieces are clear (roughly 3 times if strong color).
4. Remove solvent and shrink gel pieces with 200µL ACN, shake until gel pieces become a whitish chip (approx 15 min). Remove ACN. Dry in speed vac ~ 5 min.
5. Add 50µL Urea Solution, or enough to cover the gel slices. Incubate for 15 min at 37°C in ThermoMixer.
6. Add $1/10^{th}$ volume of IAA based on volume used in step 5 (5µL of IAA if you used 50µL of Urea solution). Incubate an additional 15 min at 37°C in water bath (keep dark).
7. Remove excess liquid. Wash gel pieces 3 times with 1mL aliquots of 50mM ABC and remove solvent after each wash.
8. Shrink with 200µL of ACN, shake for 2 min. Remove ACN. Swell in 200µL of 50mM ABC, shake for 2 min. Remove ABC.
9. Shrink with 200µL of ACN, shake for 2 min. Remove ACN. Swell in 200µL of 50mM ABC, shake for 2 min. Remove ABC.
10. Shrink with 200µL of ACN, shake for 2 min. Remove ACN, dry in speed vac ~ 5 min. While sample is in speed vac, prepare Trypsin Solution.
11. Remove dried pieces from speed vac and immediately add enough Trypsin Solution to cover the gel pieces (approx 20-40µL).
12. Shake and let rehydrate for 10 min. If needed after the 10 min, add additional Trypsin Solution to the gel pieces in order to cover them completely.
13. Incubate in the 37°C ThermoMixer overnight.

Day 2

Prepare the following Solutions:

- Neat HAc (Acetic Acid)
- 2% ACN/ 1% HAc: 20µL of ACN, 10µL of HAc, and 970µL of H_20

Procedure

1. Following overnight digestion, centrifuge the gel pieces for 5 min at 10,000g.
2. After centrifugation, transfer 50µL of the supernatant (which contains the tryptic peptides) to a MS sample vial using gel loader tips.
3. Add 100µL of the 2% ACN / 1%HAc solution to the gel pieces, shake for 30 min.
4. Centrifuge the gel pieces for 5 min at 10,000g. After centrifugation, transfer the supernatant of the gel pieces to the MS vial which already contains the supernatant from step 2 (approx 100µL of the supernatant with ~ 150µL total in MS vial).
5. Speed vac to dryness and freeze until MS analysis.
6. On the day of analysis, dissolve the dried sample with 15µL of the 2% ACN / 1% HAc solution. Shake for 5 min then centrifuge for 5 min at 10,000g.
7. See one of the MS facility members to have the sample put into the queue.

Please provide the protein sequences and/or accession numbers of the protein(s) you wish to identify. Advise the MS facility of any known modifications to the protein(s). A global database search can be performed if the protein is unknown.

Samples are placed in a queue upon receipt. A completed requisition form must be provided along with the samples. Results will be provided by email and/or PDF attachment using the email address provided.