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Discover Proteomics Operation Quick Reference Guide

Method Programming

1. Press the Open Folder button.
2. Select “New Method”.
3. Press the right arrow key until “Mode = SPS”. Press ENTER.
   NOTE: The Discover must be in SPS mode to use the Proteomics System attachment.
4. Set Power, then press ENTER. (A good starting power is 50 W. The power should be set such that maximum temperature is reached in approximately 4 minutes.)
5. Set Maximum Temperature to 55 °C, then press ENTER.
6. Set Method Run Time, then press ENTER. (A good starting run time is 15 minutes.)
7. Set Delta Temperature to 1 °C, then press ENTER.
8. Set Stirring to “HI”, then press ENTER.
9. Set Cooling to “ON”, then press ENTER.
10. Set “Next Stage = (N)”, then press ENTER.
11. Set “Save Method = (Y)”, then press ENTER.
12. Create a method name using the arrow keys, highlight “Exit”, and press ENTER. The method is now saved in the software.

Instrument Operation

1. Create a method as outlined above, or load an existing method (press the Open Folder button and use the arrow keys to scroll to the desired method, then press ENTER).
2. Ensure system is in Open Vessel Mode.
   a. Press EDIT.
   b. Scroll over using arrow keys until screen says “Open Vessel” and then press ENTER.
   c. Use the right arrow key to set “Run Open Vessel: Yes” and then press ENTER.
3. Add 25 mL of either deionized or tap water to the vessel holder bottom bath. Then add a small magnetic stir bar to the bath. Lock the vessel holder top in place over the bath.
4. Insert desired microcentrifuge sample tubes (see reverse side for suggested sample preparation protocols) into the top of the holder. Place the temperature control sample (see below for more information) in one of the two center positions.
5. Remove the attenuator from the Discover and place the assembled vessel holder into the microwave cavity. Thread the fiber optic probe through the attenuator opening, and insert it into the temperature control vessel. Ensure that the tip of the probe is below the solution level in the temperature control vessel. Lock the attenuator back in place.
6. Press the Play button to run your microwave method as programmed.

Suggested Parameters for Enzymatic Digestion

Power = 50 W	Time = 15:00
Power Mode = SPS	Stirring = Yes: High
Temperature = 55 °C	Air Cooling = 5 psi
Delta Temperature = 1 °C	Bath Solution = 25 mL water
Temperature Control = Fiber optic	Enzyme/Protein ratio = 1:100 to 1:10

Temperature Control Sample: Puncture a hole in the top of a microcentrifuge tube. Fill with the buffer solution used for the digestions. Place the temperature control sample into one of the two center holes on the vessel holder. ***You MUST use the buffer solution as the temperature control sample to ensure accurate heating.***

Consumable Parts

Part Number	Product Name
169175	5 mL Microcentrifuge Tubes (50/PK)
162810	Micro Stir Bars (50/PK)

Recommended Protocols for Enzymatic Digestion

(adapted from the protocols used by Peter Yau at the University of Illinois)

Before starting, ensure that all surfaces have been cleaned with methanol/water, including the outside of all tubes, the outside and inside of the Speed Vac and centrifuge, tube racks, bottles, razor blades, etc.

Prepare the following solutions:

- 25 mM Ammonium Bicarbonate (NH_4HCO_3): 100 mg NH_4HCO_3 per 50 mL water
- Reduction Solution: 10 mM dithiothreitol (DTT) in 25 mM NH_4HCO_3 (10 µL 1M DTT to 1 mL 25 mM NH_4HCO_3, prepare fresh daily)
- Alkylation Solution: 55 mM iodoacetamide in 25 mM NH_4HCO_3 (prepare fresh daily)
- For In-Gel Digestion Only: Destain/Dehydration Solution: 25 mM NH_4HCO_3 in 50% Acetonitrile (ACN)
- Extraction Solution: 50% ACN / 5% formic acid

In-Gel Digestion

1. Crush gel slice with a clean pipet tip or pestel.
2. For Silver stained slices, the gel must be destained (Gharahdaghi et al. 1999). For Coomassie Blue stained gel slices, proceed to step 3.
3. Add ~100 µL (or enough to cover the gel slice) of the Destain/Dehydration Solution and vortex for 10 min.
4. Using pipet tip, remove the supernatant and discard. Do not use a water aspirator or vacuum pump for this step!
5. Repeat steps 3 and 4 once.
6. Speed Vac the gel pieces to complete dryness (~20 min).
7. Add 25 µL of the Reduction Solution (prepared fresh) to each dried gel sample. Incubate in the microwave, 50 W power, at 55 °C for 10 minutes.
8. Remove supernatant, add 100 µL 25 mM NH_4HCO_3, and rinse once.
9. Add 50 µL of the Alkylation Solution to the sample. Incubate at room temperature in the dark for 1 hour.
10. Remove supernatant. Rinse sample with 100 µL 25 mM NH_4HCO_3 followed by 2 rinses with 100 µL Destain/Dehydration Solution.
11. Open a fresh vial of Promega Trypsin (20 µg), and add 0.8 mL 25 mM NH_4HCO_3. Open a fresh vial of RapiGest (Waters), add 0.8 mL 25 mM NH_4HCO_3. Combine the contents of the RapiGest to the Trypsin to give a final concentration of 25 ng/µL Trypsin and 0.0625% RapiGest.
12. Add 25 µL Trypsin/RapiGest solution to sample. Cover with additional 25 mM NH_4HCO_3 to cover the entire sample if needed. Gently vortex and give the sample a quick spin.
13. Digest in microwave with 50W at 55° C for 15 min.
14. Add 100 µL Extraction Solution to sample. Sonicate 30 minutes. Remove supernatant.
15. Repeat step 14 two more times, and pool all three extractions. Speed Vac the combined extractions to dryness.
16. Dissolve the dried peptides with 10 µL 5% ACN / 0.1% formic acid. Sample can be used for MALDI or ESI analysis.

In-Solution Digest

NOTE: For digesting in solution, samples should be free from salt and buffer (no more than 25 mM Tris or 50 mM NaCl, etc.). We DO NOT recommend performing the digestion in Tris buffer due to the change in pH with elevated temperature, which may cause diminished sequence coverage as well as lowered Mascot scores.

1. Add 20 µL of the Reduction Solution (prepared fresh) to each sample. Incubate in the microwave, 50 W power, at 55 °C for 10 minutes.
2. Add 20 µL of the Alkylation Solution to the sample. Incubate at room temperature in the dark for 1 hour.
3. Use Genotech's Perfect Focus (product number 786-124) to remove salt, buffer, DTT, and iodoacetamide. Follow Genotech's instructions to the precipitation step with Orgasol. After the sample has been in the freezer for 30 minutes, spin the protein sample from the Orgasol buffer. Resuspend protein in 25 mM NH_4HCO_3 to a concentration around 1-2 µg protein per sample.
4. Open a fresh vial of Promega Trypsin (20 µg), and add 0.8 mL 25 mM NH_4HCO_3. Add 10-25 µL Trypsin solution to the samples.
5. Digest in microwave with 50 W at 55 °C for 15 min.
6. Add 200 µL Extraction Solution to sample. Sonicate 30 minutes. Remove supernatant.
7. SpeedVac the extraction to dryness.
8. Dissolve the dried peptides with 10 µL 5% ACN / 0.1% formic acid. Sample can be used for MALDI or ESI analysis.

References:

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Hellman, U., Wernstedt, C., Gonez, J., and Heldin, C. H. (1995) Anal. Biochem. 224, 451-455.

Gharahdaghi, F., Weinberg, C. R., Meagher, D. A., Imai, B. S., and Mische, S. M. (1999) Electrophoresis 20, 601-605.