SOP: SP021

In-gel Digestion of Proteins

Materials and Reagents:
1. Coomassie stained gel containing protein of interest
2. Razor blade
3. Glass plate
4. 0.65 ml Deplasticized Eppendorf tubes (note 1)
5. 37°C incubator
6. Vortexer
7. Savant Speed-Vac
8. Trifluoroacetic acid (TFA) (10% solution)
9. Destain solution (60% acetonitrile in 0.2 M ammonium bicarbonate)
10. Extraction solution (60% acetonitrile in water, 0.1% TFA)
11. Modified trypsin sequencing grade (Roache Molecular Biochemicals, catalog # 1 418 025) (note 2)
12. Burdick and Jackson Water (note 3)
13. 0.2 M ammonium bicarbonate
14. Buffer A: 95% water, 5% acetonitrile, 0.1% acetic acid
15. Autosampler vials with lids

Protocol:
1. _____ From a Coomassie stained 1-D or 2-D polyacrylamide gel, excise protein spots of interest with a razor blade.
2. _____ Cut each spot into small pieces (~1 mm by 1 mm) and place the pieces in a deplasticized Eppendorf tube.
3. _____ Destain by covering the gel pieces with destain solution and incubating at 37°C for 30 minutes.
4. _____ Discard the destain solution and repeat up to three times until the gel is completely destained.
5. _____ Dry the gel pieces in the Savant Speed-Vac (note 4).
6. _____ Dissolve 25 µg of trypsin in 300 µl of 0.2 M ammonium bicarbonate (note 5).
7. _____ Add trypsin solution to the gel slices so that the gel slices are covered (generally 3 to 5 µl).
8. _____ Incubate at room temperature until the trypsin solution is completely absorbed by the gel slices (~15 min)
9. _____ Add 0.2 M ammonium bicarbonate in 10-15 µl increments to completely reswell the gel (note 6).
10. _____ Incubate the gel slices overnight at 37°C.
11. _____ Terminate the reaction by adding 1/10th the digest volume of 10% TFA.
12. _____ Collect the supernatant, and place it in a new deplastiziced tube.
13. _____ Add 100 µl of the extraction solution to the gel slices and vortex.
14. _____ Incubate the extract solution and gel slices at 37°C for 40 min (note 7).
15. _____ Centrifuge the extract, collect the supernatant and add it to the extract collected in step 12.
16. _____ Repeat steps 13 to 15.

17. _____ Place the combined extract in the Savant Speed-Vac and dry (note 8).

18. _____ Add 20 μl of buffer A to the sample and mix well.

19. _____ Centrifuge the sample for 5 minutes.

20. _____ Pipet the sample out of the tube, being sure not to touch the bottom of the tube with the pipet tip (there will be a small volume left in the tube).

21. _____ Pipet sample into an autosampler vial, being careful not to introduce any bubbles into the vial.

22. _____ Place the cap on the autosampler vial and label the vial.

23. _____ Store the samples at –20°C until analysis by LC-MS-MS.

Notes:
1. Eppendorf tubes are deplasticized by filling with 60% acetonitrile-0.1% TFA, followed by mixing, incubation at room temperature for 1 hour and decanting of the solution. This process is repeated two times for each tube and the tubes are dried in the Savant Speed-Vac.
2. This procedure can be used with proteases other than trypsin, however the buffer for the digestions may differ for other proteases.
3. All solutions should be made using burdick and jackson water.
4. See SOP SP005 for use of the savant.
5. When preparing a new vial of trypsin, be sure to date and initial it. The reconstituted trypsin should be stored at 4°C and can be used for up to two weeks.
6. Allow 10-15 minutes for swelling of the gel pieces between additions of ammonium bicarbonate. The final volume should just cover the tops of the gel pieces.
7. After addition of the extract solution, vortex the tubes and put them in the incubator for 20 minutes, vortex again, then back in the incubator for 20 more minutes.
8. Do not allow to dry completely. Leave 1-2 μl of liquid in the bottom of the tube.

References: