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Central Proteomics Facility
Tryptic Digest Protocol

The facility can perform digests for you (at a charge), or can provide the chemicals, equipment and facilities for you to perform them in the facility.

Solutions required:

Solution B: 0.04 g ammonium bicarbonate (ammonium hydrogen carbonate) in 10 mL Milli-Q grade water and 10 mL HPLC grade acetontrile.
Acetonitrile: HPLC grade.
25 mM ammonium bicarbonate: 0.04 g in 20 mL Milli-Q grade water.
10 mM DTT: 0.031 g in 20 mL in 25mM ammonium bicarbonate solution.
55 mM Iodoacetamide: 0.2 g in 20 mL ammonium bicarbonate solution.

Promega Sequencing Grade Modified Trypsin (catalogue number: V5111. How to reconstitute the trypsin: to 1 vial add 100 uL Promega resuspension buffer. Store in 5 uL aliquots at -20 C until use. Prior to use add 95 uL 25 mM ammonium bicarbonate – the tryspin in now active. Any trypsin remaining after use should be disposed of.

Extraction buffer: 10 mL Milli-Q grade water + 10 mL acetonitrile + 20 microlitres formic acid.

Protocol

1) Cut-out gel bands on a clean surface with a clean scalpel. Dice into 1mm cubes and place in tube.
2) Wash bands with 100 uL Solution for 30 mins then discard supernatant

3) Repeat Wash with 100 uL Solution B 30 mins then discard supernatant (repeat until bands are no longer a strong blue colour).
4) Wash in 100 uL 100% acetontrile for 10 mins and remove (gel pieces should dehydrate and go white).
5) Dry in SpeedVac for 10 mins or remove cap for 10 mins (all acetonitrile should have evaporated).
6) Add 100 uL 10 mM DTT for 30 minutes at 37 C and discard.
7) Wash gel pieces with 25mM ammonium bicarbonate solution and discard.
8) Wash gel pieces with 25mM ammonium bicarbonate solution and discard.
9) Wash in acetonitrile until gel pieces are white and discard.
10) Add 100 uL 55 mM Iodoacetamide for 60 mins (IN DARK) and discard
11) Wash bands with 100 uL Solution B for 10 mins and discard.
12) Wash bands with 100 uL Solution B for 10 mins and discard.

10) Wash in 100 uL100% acetontrile until gel pieces are white, then discard.
11) Dry in SpeedVac for 10 mins or remove cap for 10 mins.
12) Add 20 uL trypsin to each tube.
13) Digest overnight at 37 C.
14) Add 1 uL of formic acid to stop digest.
15) Remove supernatant containing peptides to clean new tube.
16) Add 50uL of extraction buffer to gel piece and incubate for 30 mins.
17) Remove supernatant containing peptides and pool with existing supernatant.
18) SpeedVac to dryness (available in Central Proteomics Facility) and bring to Central Proteomics Facility ASAP.

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