

Trypsin In-gel Digestion Procedure

To avoid keratin contamination, wear gloves throughout this process.

1. Place the gel on a stringently cleaned glass plate and excise the gel band of interest out of the gel using a clean razor blade. Cut the band into the smallest pieces possible.
2. Place the gel pieces into a centrifuge tube and cover with acetonitrile for approximately 15 minutes at room temperature. Spin the tubes, remove the acetonitrile by pipette, and dry the gel pieces by Speed-Vac.
3. Re-swell the dried gel pieces in buffer containing trypsin in 50mM NH₄-HCO₃ at 4°C for 45 minutes. Add enough buffer so the gel pieces are just covered. The suggested amount of trypsin is 12.5 ng/ul of buffer for proteins that have been silver stained.
4. Incubate the swelled gel pieces in trypsin buffer at 37°C for at least 3 hours (ideally overnight).
5. Centrifuge the gel pieces and collect the supernatant.
6. Add 25mM NH₄-HCO₃ to the gel pieces and soak for approximately 25 minutes.
7. Further extract the peptides by soaking the gel pieces in 5% formic acid in 50% acetonitrile 45% water for 30 minutes, followed by spinning and collection of the supernatant. Repeat 3 times.
8. Dry the collected supernatants by Speed-Vac until the desired volume has been reached.

In-Solution Trypsin Digestion Method 1

The sample should contain a minimal amount of DTT or detergents for the following method to work.

1. By Speed-Vac, dry the protein sample in a microfuge tube.
2. Dissolve the proteins in a minimum of 20ul of freshly made 0.1M ammonium bicarbonate. Try to keep the volume of the digest solution as small as possible.
3. Denature proteins by incubating at 55°C for 30 minutes.
4. Add trypsin to the sample to a trypsin: sample ratio of 1:100.
5. Incubate at 37°C overnight.
6. Digestion can be confirmed as a disappearance of the corresponding protein band on protein SDS-PAGE.

In-Solution Trypsin Digestion Method 2

This procedure is routinely used in the lab after ICAT labeling because it allows for some DTT or detergents, though method 1 results in better digestion.

1. Dilute the sample to a maximal detergent concentration of 0.01% SDS.
2. Add trypsin to a trypsin: sample ratio of 1:20.
3. Incubate at 37°C overnight.
4. Digestion can be confirmed as a disappearance of the corresponding protein band on protein SDS-PAGE.

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