

IN-GEL TRYPTIC DIGEST FOR LC-ESI MS

1. Ensure gel pieces are at neutral pH
2. Add 10 μ L of 45 mM dithiothreitol (DTT) and 100 μ L of 100 mM TRIS pH 7.8; incubate at 55° for 30 minutes.
3. Discard solution phase.
4. Add 10 μ L of 100 mM acrylamide and 100 μ L of 100 mM Tris pH 7.8, incubate at room temperature for 30 minutes. (Alternatively, substitute 10 μ L of 100mM iodoacetamide for the acrylamide and incubate at room temperature for 1 hour in the dark.)
5. Discard solution phase.
6. Wash gel pieces with 500 μ L of 50 mM Tris pH 7.8 / 50% acetonitrile for 30 minutes.
7. Discard solution phase and dry gel to completion in SpeedVac.
8.
 - a. For gel bands or large spots, add 5 pmol trypsin in 25 mM Tris pH 7.8, plus a minimal amount of 25 mM Tris pH 7.8, to allow gels to swell. When gels are fully swollen, add sufficient 25 mM Tris pH 7.8 to just cover the gel.
 - b. For spots from 2-D gels, use 2.5 pmol of trypsin and a minimal amount of 25 mM Tris pH 7.8. Typically 10-20 μ L total liquid will give the optimal result of fully swollen gels plus 5-10 μ L of free solution.
9. Incubate overnight at 37° C.
10. For LC-MS/MS analysis, the solution phase is removed and injected without further treatment.

REAGENTS

DTT	Sigma	D-5545	FW 154.2 g/mol	mL = mg/6.93
Acrylamide	ICN	814320	FW 71.08 g/mol	mL = mg/7.11
Trypsin	Promega	V5111		