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Protein Sample Preparation from Acrylamide Gel for Mass Spectrometry

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We use this protocol and it's working

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Abstract

This protocol details how to prepare protein samples from polyacrylamide gels for mass spectrometry proteomics.

Materials

Digestion Buffer:

	A	B
	Tris-HCl	25 mM
	Acetonitrile	10%
	Trypsin	10 ng µl–1

Protein Sample Preparation from Acrylamide Gel

1 Slice the protein band of interest from a Coomassie blue-stained polyacrylamide gel using a scalpel.

> Note
>
> Avoid contamination by human keratin by using clean apparel and reagents. Wear gloves.

2 Wash thoroughly the gel pieces with 150 µL of de-staining buffer ([M] 25 millimolar (mM) ammonium bicarbonate, 50% ethanol).

3 Dehydrate the gel pieces with 150 µL of pure ethanol.

4 Remove the ethanol and dry the gel pieces using a SpeedVac.

5 Add 50 µL of digestion buffer (25 mM Tris-HCl, 10% acetonitrile, 10 ng μl^{-1} trypsin).

Digestion Buffer:

	A	B
	Tris-HCl	25 mM
	Acetonitrile	10%
	Trypsin	10 ng µl–1

6 Cool On ice for 00:20:00 . 20m

7 Add 50 µL of [M] 25 millimolar (mM) ammonium bicarbonate buffer.

8 Incubate Overnight at 37 °C . 8h

9 Collect the peptides released to the supernatant.

10 Extract additional peptides by incubation at 25 °C for 00:20:00 in 100 µL of extraction buffer (3% trifluoroacetic acid (TFA), 30% acetonitrile). 20m

11 Centrifuge at 4000 rpm, 00:02:00 . 2m

12 Collect the supernatant. Repeat steps 10-12 twice.

13 Dehydrate the gel pieces with 100 µL of pure acetonitrile at 25 °C during 00:10:00 . 10m

14 Centrifuge at 4000 rpm, 00:02:00 . 2m

15 Collect the supernatant and combine with previous supernatants.

16 Remove the acetonitrile using a SpeedVac.

17 Add 70 µL of [M] 2 Mass Percent Tris-HCl pH 8.0, [M] 10 millimolar (mM) TCEP, and [M] 40 millimolar (mM) chloroacetamide (CAA).

18 Incubate 00:30:00 at 37 °C . 30m

19 Acidify the peptides to 1% TFA.

20 For the desalting of the peptides, prepare a SDB-XC stage tip by pushing 3 plugs of 0.8 mm diameter from C18/SDB-XC and push them into a 300 µl pipette tip.

21 Wash with 20 µL of methanol to complete dryness by spinning.

22 Wash with 20 µL of buffer B (0.1% Formic acid and 80% acetonitrile in water). Spin the tips but make sure that the tip is not dry. A small volume of buffer B should be visible on the top.

23 Equilibrate the tips 2 times with 200 µL of buffer A (0.1% formic acid in water).

24 Load the sample onto the stage tips and spin at 1500 x g .

25 Wash the tip with 100 µL of buffer A and spin to dryness.

26 Elute the peptides from the tip using 60 µL of buffer B.

27 After elution, speed-vac the samples at 30 °C for 00:40:00 and store at -20 °C until loading on the LC-MS system. 40m