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Gel Slice Sample Preparation for Proteomics

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2 Works for me dx.doi.org/10.17504/protocols.io.bgj3juqn

LBNL-omics

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ABSTRACT

This protocol details steps for gel slice sample preparation for proteomic data acquisition. It was adapted from Shevchenko et al. "In-gel digestion for mass spectrometric characterization of proteins and proteomes."*Nature protocols* 1.6 (2006): 2856.

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MATERIALS

NAME	CATALOG #	VENDOR
Eppendorf tubes 1.5 mL uncolored	022363204	Eppendorf Centrifuge
DTT	DTT-RO	Millipore Sigma
Iodoacetamide	I1149	Millipore Sigma
Water LC-MS grade B&J Brand	BJLC365-2.5	VWR Scientific
Ammonium Bicarbonate LC-MS grade	BJ40867-50G	VWR Scientific
Acetonitrile LC-MS grade B&J Brand	BJLC015-2.5	VWR Scientific
Sequencing Grade Modified Trypsin Porcine	V511A	Promega

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NAME	CATALOG #	VENDOR
Acetic Acid	818755	Millipore Sigma
Formic Acid	28905	Thermo Fisher Scientific
Eppendorf ThermoMixer C	2231000667	pipette.com
Eppendorf Vacufuge Concentrator	07-748-13	Fisher Scientific

SAFETY WARNINGS

Wear proper PPE (gloves, safety goggle, and lab coat), and prepare solvents in a chemical fume hood.
Store organic solvents in a flammable storage cabinet when not in use.
Discard used solvents and buffers in appropriate waste containers.

Buffer Preparation 30m

1

Buffers to prepare fresh:

100 ml of 100 mM Ammonium Bicarbonate (AMBIC):	0.79 g Ammonium Bicarbonate (VWR Scientific, Cat.#BJ40867-50G) 100 ml LC-MS grade Water (VWR Scientific, Cat.#BJLC365-2.5)
1 ml of 50mM Ammonium Bicarbonate/Acetonitrile (AMBIC/ACN):	250 µl 100 mM Ammonium Bicarbonate 250 µl LC-MS grade Water 500 µl Acetonitrile (VWR Scientific, Cat.#BJLC015-2.5)
1 ml of 10 mM 1,4-Dithiothreitol (DTT), 25 mM Ammonium Bicarbonate (AMBIC):	10 µl 1M 1,4-Dithiothreitol (Millipore Sigma, Cat.#DTT-RO) 250 µl 100 mM Ammonium Bicarbonate 740 µl LC-MS grade Water
1 ml of 55 mM Iodoacetamide, 25 mM Ammonium Bicarbonate (IAA/AMBIC):	10 mg Iodoacetamide (Millipore Sigma, Cat.#I1149) 250 µl 100 mM Ammonium Bicarbonate 750 µl LC-MS grade Water
0.200 ml of 100 ng/ul of Trypsin:	20 µg Sequencing Grade Modified Trypsin (Promega, Cat.#V511A) 200 µl 50 mM Acetic Acid Millipore Sigma, Cat.#818755)
0.100 ml of 12.5 ng/ml Trypsin/50 mM Ammonium Bicarbonate (Trypsin/AMBIC):	12.5 µl 100 ng/ul Trypsin 50 µl 100 mM Ammonium Bicarbonate 37.5 µl LC-MS grade Water
1 mL of 50% Acetonitrile/5% Formic Acid (ACN/FA):	0.5 ml Acetonitrile 0.05 ml Formic Acid (Thermo Fisher Scientific, Cat.#28905) 0.45 ml LC-MS grade Water
1mL of Buffer A	0.001 ml Formic Acid 1 ml Water

Store DTT in **-20 °C** and Trypsin in **-80 °C** .

IAA is light sensitive. Store in amber tube (Fisher Scientific, Cat.#05-402-31).

Day 1: Washing the gel slices 1h 15m

2 15m

Wash gel slice with **150 µl Water** in a tube (Eppendorf Centrifuge, Cat.#022363204). Incubate for **00:15:00** then remove liquid.

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3 15m

Wash gel slice with **150 µl 50 mM AMBIC/ACN** and incubate for **00:15:00** then remove liquid.

Discard used buffers and solvents in appropriate waste containers.

4 15m

Add **50 µl Acetonitrile** and incubate for **00:15:00** then remove liquid.

5 20m

Add **100 µl 50 mM AMBIC/ACN** and incubate for **00:20:00** then remove liquid.

6 10m

Add **50 µl Acetonitrile** to cover gel slice. The gel slice will visibly shrink and turn white. Incubate for **00:05:00** to **00:10:00** then remove liquid.

Day 1: Trypsin digestion 3h

7 1h

Add **50 µl 10 mM DTT, 25 mM AMBIC** and incubate in **56 °C water bath** for **01:00:00** then remove liquid.

8 45m

Add **50 µl 55 mM IAA, 25 mM AMBIC** and incubate in the dark, at **Room temperature** for **00:45:00** then remove liquid.

9 15m

Wash gel slice by adding **50 µl 50 mM AMBIC/ ACN** and incubating for **00:15:00** then remove liquid.

10

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Repeat wash step 9.

11 5m

Add **50 µl Acetonitrile** to cover gel slice. The gel slice will visibly shrink and turn white. Incubate for **00:05:00** to **00:10:00** then remove liquid.

.

12 30m

Add **10 µl 12.5 ng/ml Trypsin, 50 mM AMBIC** and incubate on **On ice** for **00:30:00** , then remove excess liquid, if necessary.

All of the liquid (trypsin) should be absorbed into the gel slice at the end of 30 minutes. The gel slice will look clear/hydrated again. There is usually no liquid leftover because only a small amount of trypsin is added however, if necessary, remove excess trypsin before overnight digestion.

13 15m

Add **50 Milimolar (mM) AMBIC** to cover the gel slice.

Wait 15-20 minutes to make sure the gel slice is still completely covered in liquid. Sometimes the gel is not completely rehydrated after the trypsin has been absorbed, and it absorbs a lot of the AMBIC if you only use enough to cover. This is especially a problem with larger gel pieces.

14 16h

Digest the protein(s) at **37 °C** **Overnight** in an incubator or thermocycler.

Day 2: Recovering digested peptides 4h

15 Remove gel slice from **37 °C** incubator or thermocycler.

16 15m

Add **20 µl 50 mM AMBIC** after tryptic digestion. Incubate at **Room temperature** for **00:15:00** .

17

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Collect liquid in a clean tube.

18

Add **30 µl 50% ACN/ 5% FA** to cover the gel slice.

19 20m

Incubate for **00:20:00** on a benchtop shaker (Pipette.com, Cat.#2231000667) set at **Room temperature** .

20

Collect liquid.

21

Repeat steps 18-20.

22 5m

Add **20 µl Acetonitrile** . Incubate for **00:05:00** then collect liquid.

23

Use a SpeedVac (Fisher Scientific, Cat.#07-748-13) to dry the liquid completely.

Day 2: Sample Prep For MS 5m

24

After the tubes are completely dry in Step 24, add **17 µl Buffer A** then transfer to plastic autosampler vials (Agilent, Cat.#5182-0567,#5182-0564) or 96-well plate (Bio-Rad, Cat.#HSP9655).

25

Store at **-20 °C** until ready for LC-MS/MS analysis.

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