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## © Gel Slice Sample Preparation for Proteomics

Jennifer Gin<sup>1</sup>, Leanne Chan<sup>1</sup>, Christopher Petzold<sup>1</sup>

<sup>1</sup>Lawrence Berkeley National Laboratory

2 Works for me

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LBNL-omics

Jennifer Gin

**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.

EXTERNAL LINK

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## MATERIALS

NAME	CATALOG #	VENDOR
Eppendorf tubes 1.5 mL uncolored	022363204	Eppendorf Centrifuge
DTT	DTT-RO	Millipore Sigma
lodoacetamide	11149	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	0.79 g Ammonium Bicarbonate (VWR Scientific, Cat.#BJ40867-50G)
Bicarbonate (AMBIC):	100 ml LC-MS grade Water (VWR Scientific, Cat.#BJLC365-2.5)
1 ml of 50mM Ammonium	250 µl 100 mM Ammonium Bicarbonate
Bicarbonate/Acetonitrile	250 μl LC-MS grade Water
(AMBIC/ACN):	500 μl Acetonitrile (VWR Scientific, Cat.#BJLC015-2.5)
1 ml of 10 mM 1,4-Dithiothreitol	10 µl 1M 1,4-Dithiothreitol (Millipore Sigma, Cat.#DTT-RO)
(DTT), 25 mM Ammonium	250 µl 100 mM Ammonium Bicarbonate
Bicarbonate (AMBIC):	740 µl LC-MS grade Water
1 ml of 55 mM lodoacetamide, 25	10 mg Iodoacetamide (Millipore Sigma, Cat.#I1149)
mM Ammonium Bicarbonate	250 µl 100 mM Ammonium Bicarbonate
(IAA/AMBIC):	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	12.5 µl 100 ng/ul Trypsin
Trypsin/50 mM Ammonium	50 μl 100 mM Ammonium Bicarbonate
Bicarbonate (Trypsin/AMBIC):	37.5 μl LC-MS grade Water
1 mL of 50% Acetonitrile/5%	0.5 ml Acetonitrile
Formic Acid (ACN/FA):	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  $\ \mbox{\$ -20 °C}$  and Trypsin in  $\ \mbox{\$ -80 °C}$  .

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

Day 1: Washing the gel slices

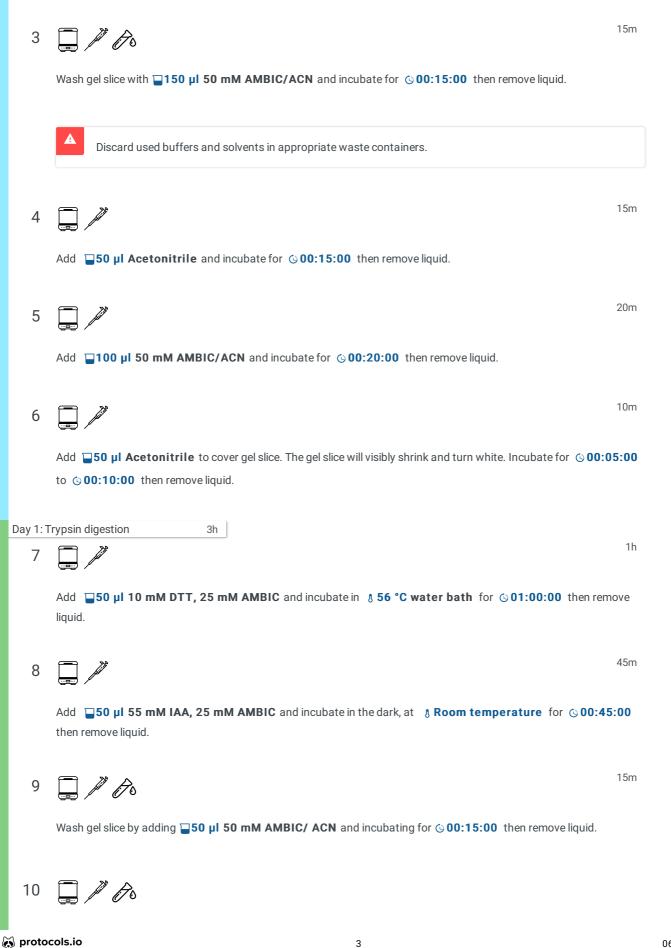
1h 15m

2

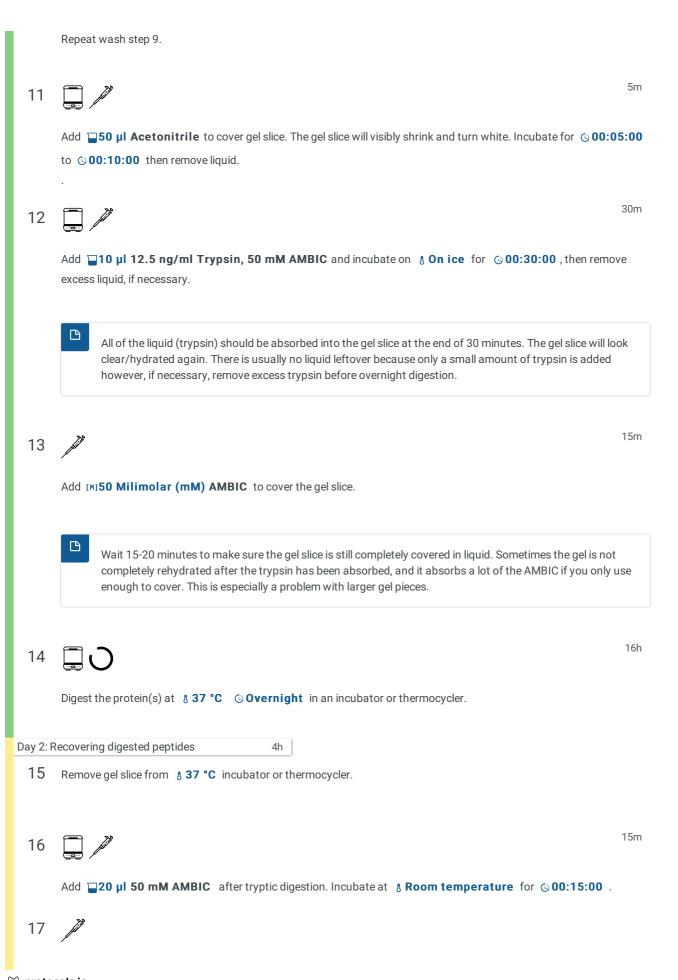


15m

Wash gel slice with  $\Box$ 150  $\mu$ l Water in a tube (Eppendorf Centrifuge, Cat.#022363204). Incubate for  $\bigcirc$  00:15:00 then remove liquid.



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Collect liquid in a clean tube. 18 Add 30 µl 50% ACN/ 5% FA to cover the gel slice. 19 Incubate for © 00:20:00 on a benchtop shaker (Pipette.com, Cat.#2231000667) set at 8 Room temperature.

20m

20

Collect liquid.

21

Repeat steps 18-20.

5m 22

Add  $\blacksquare 20~\mu l$  Acetonitrile . Incubate for  $\circlearrowleft 00:05:00$  then collect liquid.

23

25

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

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Day 2: Sample Prep For MS 5m 24

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

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

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