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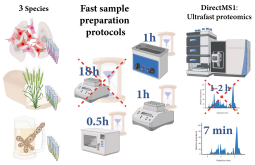
DirectMS1 Ultrafast proteomics: Protein extraction & digestion



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From Days to Minutes: Accelerated Protein Digestion for Fragmentation-Free Ultrafast Mass Spectrometry
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We use this protocol and it's working

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Keywords: ultrafast proteomics, microwave digestion, accelerated protein digestion, fragmentation-free mass spectrometry, proteomics, sample preparation, directms1 ultrafast proteomic, digestion ultrafast proteomic workflow, throughput analysis in clinical proteomic, clinical proteomic, bottleneck of overnight protein digestion, high reproducibility in protein quantitation, overnight protein digestion, protein extraction, protein quantitation, rapid sample preparation, trypsin digestion, protein, mass spectrometry, yeast quantitation standard, reducing preparation time, employing liquid chromatography, preparation time, liquid chromatography

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Abstract

Ultrafast proteomic workflows, employing liquid chromatography-mass spectrometry, demand equally rapid sample preparation. To overcome the bottleneck of overnight protein digestion, this study optimized reduction, alkylation, and trypsin digestion using ultrasound, microwave irradiation, and elevated temperatures, achieving completion within 30-60 minutes. Validation using various sample types (S. cerevisiae, A549 cells, winter wheat) and human-yeast quantitation standards confirmed the reproducibility of label-free quantification. The microwave-assisted method, reducing preparation time 35-fold, showed high reproducibility in protein quantitation, demonstrating the potential for high-throughput analysis in clinical proteomics.

Guidelines

For troubleshooting help, feel free to message to the corresponding author iatarasova@gmail.com.

Avoid salts and non-volatile buffers in your final purification steps, if possible. In most cases,dry your sample down in a speed-vac and re-suspend it in 50mM ammonium bicarbonate pH 8.3 for a tryptic digestion.

The list of reagents can be found in the 'Materials' section

Materials

	A
	Acetonitrile LC-MS
	Ammonium bicarbonate (NH4HCO3)
	MilliQ water
	SDS
	Dithiothreitol
	Iodoacetamide (single-use vial 56mg)
	ProteaseMAX(TM) Surfactant Trypsin Enhancer, 1mg
	Pierce BCA Protein Assay Kit
	Methanol
	Chloroform
	Urea
	Sequencing Grade Modified Trypsin
	Copure‰ C18 Endcapped Octadecyl
	Trifluoroacetic acid

Protocol materials

- Pierce BCA Protein Assay Kit **Thermo Fisher Scientific Catalog #**23225
- Pierce BCA Protein Assay Kit **Thermo Fisher Scientific Catalog #**23225
- Methanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M3641
- Chloroform
- Pierce BCA Protein Assay Kit **Thermo Fisher Scientific Catalog #**23225
- Urea **Merck MilliporeSigma (Sigma-Aldrich) Catalog #** 57-13-6
- SDS **Bio-Rad Laboratories Catalog #**1610301
- Dithiothreitol **neoFroxx Catalog #** 3483-12-3
- Iodoacetamide (single-use vial 56mg) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A3221
- Acetonitrile LC-MS **Biosolve Catalog #**01207802
- Ammonium bicarbonate (NH4HCO3) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**AC393212500
- ProteaseMAX(TM) Surfactant Trypsin Enhancer, 1mg **Promega Catalog #**V2071
- Acetonitrile LC-MS **Biosolve Catalog #**01207802
- Ammonium bicarbonate (NH4HCO3) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**AC393212500
- ProteaseMAX(TM) Surfactant Trypsin Enhancer, 1mg **Promega Catalog #**V2071
- Sequencing Grade Modified Trypsin **Promega Catalog #**V5111
- Dithiothreitol **neoFroxx Catalog #** 3483-12-3
- Iodoacetamide (single-use vial 56mg) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A3221
- Sequencing Grade Modified Trypsin **Promega Catalog #**V5111
- Dithiothreitol **neoFroxx Catalog #** 3483-12-3
- Iodoacetamide (single-use vial 56mg) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A3221
- Sequencing Grade Modified Trypsin **Promega Catalog #**V5111
- Dithiothreitol **neoFroxx Catalog #** 3483-12-3
- Iodoacetamide (single-use vial 56mg) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A3221
- Dithiothreitol **neoFroxx Catalog #** 3483-12-3
- Sequencing Grade Modified Trypsin **Promega Catalog #**V5111
- Copure® C18 Endcapped Octadecyl **Biocomma**
- Copure® C18 Endcapped Octadecyl **Biocomma**
- Copure® C18 Endcapped Octadecyl **Biocomma**
- MilliQ water

Methanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M3641

Copure® C18 Endcapped Octadecyl **Biocomma**

Copure® C18 Endcapped Octadecyl **Biocomma**

Urea **Merck MilliporeSigma (Sigma-Aldrich) Catalog #** 57-13-6

Trifluoroacetic acid (TFA)

Iodoacetamide (single-use vial 56mg) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A3221

Sequencing Grade Modified Trypsin **Promega Catalog #**V5111

Trifluoroacetic acid (TFA)

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Trifluoroacetic acid (TFA)

Trifluoroacetic acid (TFA)

Troubleshooting

Safety warnings

To ensure safety, reactions with chloroform, methanol and trifluoroacetic acid must be carried out in a fume hood. The experimenter must be protected with gloves throughout the experiment.

Ethics statement

The authors declare no competing ethics statement.

Yeast protein extraction

2h

1

5m

Sample 1*10^9 yeast cells.

Add 100 µL Lysis buffer volume to the Sample

Lysis buffer:

[M] 10 % volume

Acetonitrile LC-MS **Biosolve Catalog #**01207802

[M] 50 millimolar (mM)

Ammonium bicarbonate (NH4HCO3) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**AC393212500

[M] 0.1 Mass / % volume

ProteaseMAX(TM) Surfactant Trypsin Enhancer, 1mg **Promega Catalog #**V2071

2

1h

600 rpm, 25°C, 01:00:00

3

5m

Sonication for 2 min (10 seconds on/off cycles) at each 30, 60, 80% amplitudes on ice

0 °C

Equipment

QSonica SONICATOR SYSTEM Q800R1	NAME
Sonicator	TYPE
QSonica	BRAND
discontinued	SKU

4 15000 rcf, 00:10:00 10m

5 Collected the supernatant 5m

6 Concentration measurement 35m

Pierce BCA Protein Assay Kit **Thermo Fisher Scientific Catalog #**23225

Equipment	
SPECTROstar Omega	NAME
Microplate reader	TYPE
BMG LABTECH	BRAND
None	SKU
https://www.bmglabtech.com/en/spectrostar-omega/	LINK

A549/MCF7 protein extraction 2h 15m

7 A549 or MCF7 cells 5m

Add 100 µL Lysis buffer volume to the Sample

Lysis buffer:

[M] 10 % volume Acetonitrile LC-MS **Biosolve Catalog #**01207802

[M] 50 millimolar (mM)

Ammonium bicarbonate (NH4HCO3) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**AC393212500

[M] 0.1 Mass / % volume

ProteaseMAX(TM) Surfactant Trypsin Enhancer, 1mg **Promega Catalog #**V2071

8 600 rpm, 25°C, 01:00:00 1h

9 550 rpm, 95°C, 00:05:00 5m

10 15 minutes in ultrasonic bath 50 Hz 15m

Equipment

PSB-1335-03	NAME
Ultrasonic bath	TYPE
PCB-GALS	BRAND
1335-03	SKU

11 10000 rcf, 00:10:00 10m

12 Collected the supernatant 5m

13 Concentration measurement 35m

Pierce BCA Protein Assay Kit **Thermo Fisher Scientific** **Catalog #**23225

Equipment

SPECTROstar Omega	NAME
Microplate reader	TYPE
BMG LABTECH	BRAND
None	SKU
https://www.bmglabtech.com/en/spectrostar-omega/	LINK

Wheat protein extraction

2h 49m

14 Sample 250 mg of plant tssue 5m

15 Disrupt the Sample using 3m

Equipment

CryoMill	NAME
Retsch GmbH, Berlin, Germany	BRAND
20.749.0001	SKU

for 3 min at 30 Hz (3 cycles)

16 Add 1 mL Lysis buffer 5m

([M] 8 Molarity (M)

Urea **Merck MilliporeSigma (Sigma-Aldrich) Catalog #** 57-13-6 in

[M] 2 % volume SDS **Bio-Rad Laboratories Catalog #**1610301) to 250 mg of homogenate

17 incubate 1000 rpm, 25°C, 01:00:00 1h

18 20000 rcf, 00:15:00 15m

19 Pierce BCA Protein Assay Kit **Thermo Fisher Scientific Catalog #**23225 35m

Equipment

Microplate reader	NAME
BMG LABTECH	BRAND
no	SKU
https://www.bmglabtech.com/en/spectrostar-omega/	LINK

20 Collect supernatant 5m

21 Chloroform - methanol extraction

21.1 collect 200 µL supernatant 2m

21.2 add 800 µL 2m

Methanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M3641

21.3 add 200 µL Chloroform 2m

21.4 add 600 µL MilliQ water 2m

21.5 centrifugate 15000 rcf, 00:15:00 15m

21.6 discard supernatant over interphase 2m

21.7 add 600 µL 2m
Methanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M3641

21.8 centrifugate 7000 rcf, 00:07:00 7m

21.9 discard supernatant 2m

21.10 dissolve the pillet in [M] 2 Molarity (M) 200 µL 5m
Urea **Merck MilliporeSigma (Sigma-Aldrich) Catalog #** 57-13-6

22 Digestion protocols
Protocol #1 - overnight protein digestion (19h total time)
Protocols #2-5 - 30-60 minutes protein digestion time

STEP CASE

Protocol #1 5 steps

23 Add Dithiothreitol **neoFroxx Catalog #** 3483-12-3 up to [M] 10 millimolar (mM) 20m
500 rpm, 56°C, 00:20:00

24 Add Iodoacetamide (single-use vial 56mg) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A3221 up to [M] 20 millimolar (mM) Incubate in the dark for 00:30:00 30m

25 Add Sequencing Grade Modified Trypsin **Promega Catalog #**V5111 [M] 1/75 %(m/m) 500 rpm, 37°C Overnight 18h

26 Add Trifluoroacetic acid (TFA) up to [M] 1 % volume 5m

Desalting 20m

27 C18 desalting Copure® C18 Endcapped Octadecyl **Biocomma** 20m

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