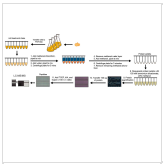


Mar 29, 2021

Chloroform-Methanol Protein Extraction with Bead Beating for Yeast

Forked from Chloroform-Methanol Protein Extraction with Zymolyase Treatment for Yeast (High Throughput)

Leanne Chan[1], Christopher Petzold[1]

[1]Lawrence Berkeley National Laboratory

1 Works for me dx.doi.org/10.17504/protocols.io.btj3nkqn

LBNL-omics

Christopher Petzold
Lawrence Berkeley National Laboratory

SUBMIT TO PLOS ONE

ABSTRACT

We adapted a high-throughput sample preparation workflow for Gram-negative bacteria to work with yeast. It consists of a bead-beating step, cell lysis, protein precipitation, protein resuspension, protein quantification, and normalization of protein concentration followed by standard bottom-up proteomic procedures of reducing and blocking cysteine residues and tryptic digestion.

This protocol was adapted from the manual sample preparation method found in Chen, Y., et al. "Automated “Cells-To-Peptides” Sample Preparation Workflow for High-Throughput, Quantitative Proteomic Assays of Microbes."*Journal of proteome research* 18.10 (2019): 3752-3761.

EXTERNAL LINK

https://pubs.acs.org/doi/abs/10.1021/acs.jproteome.9b00455

DOI

dx.doi.org/10.17504/protocols.io.btj3nkqn

EXTERNAL LINK

https://pubs.acs.org/doi/abs/10.1021/acs.jproteome.9b00455

PROTOCOL CITATION

Leanne Chan, Christopher Petzold 2021. Chloroform-Methanol Protein Extraction with Bead Beating for Yeast. **protocols.io** https://dx.doi.org/10.17504/protocols.io.btj3nkqn

FORK NOTE

FORK FROM

Forked from Chloroform-Methanol Protein Extraction with Zymolyase Treatment for Yeast (High Throughput), Christopher Petzold

KEYWORDS

Proteomics, Sample preparation, Protein quantification, Protein extraction, Trypsin digestion, Yeast, Bead-beating

Citation: Leanne Chan, Christopher Petzold (03/29/2021). Chloroform-Methanol Protein Extraction with Bead Beating for Yeast. https://dx.doi.org/10.17504/protocols.io.btj3nkqn

CREATED

Mar 23, 2021

LAST MODIFIED

Mar 29, 2021

PROTOCOL INTEGER ID

48475

GUIDELINES

- All centrifuge steps use an Eppendorf 5810R centrifuge.

- Cells were lysed in a Qiagen TissueLyser II system.

- A Molecular Devices Spectramax 250 microplate reader is used for the protein quantification assay measurement.

- Tryptic digestion is accomplished in an AB Sciex Veriti 96-well thermocycler.

Notes:
- For fewer than 30 samples PCR strips are easier to handle than plates, but once the number of samples is greater than 30 we find that a plate is a better choice.

- A multi-channel pipette is recommended for large numbers of samples.

- Measuring the amount of cells by multiplying the OD of the culture by the volume of the culture provides a good estimate for most applications, but the amount of cells can be determined more accurately from dry cell weight (DCW) or cell counting methods.

- We typically extract over 100 ug of protein from 2.0 OD*mLs of cells, so adjust the starting amount of cells for your organism or culturing conditions.

MATERIALS TEXT

MATERIALS

Corning™ 96-Well Solid Black Polystyrene Microplates (Costar 3915) **Fisher Scientific Catalog #07-200-590**

Pierce™ Bovine Serum Albumin Standard Pre-Diluted Set **Thermo Fisher Catalog #23208**

Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) **Sigma Aldrich Catalog #C4706**

Iodoacetamide **Millipore Sigma Catalog #I1149**

Methanol LC-MS grade B&J Brand **VWR Scientific Catalog #BJLC230-2.5**

Chloroform for HPLC **Sigma – Aldrich Catalog #34854**

Water LC-MS grade B&J Brand **VWR Scientific Catalog #BJLC365-2.5**

Ammonium Bicarbonate LC-MS grade **VWR Scientific Catalog #BJ40867-50G**

Citation: Leanne Chan, Christopher Petzold (03/29/2021). Chloroform-Methanol Protein Extraction with Bead Beating for Yeast. https://dx.doi.org/10.17504/protocols.io.btj3nkqn

DC Protein Assay Reagent A **Bio-rad Laboratories Catalog #500-0113**

DC Protein Assay Reagent B **Bio-rad Laboratories Catalog #500-0114**

8-strip PCR Tubes with Caps **Axygen Catalog #14-222-251**

Trypsin **Sigma Aldrich Catalog #T6567-1MG**

PCR Plate 96-well non-skirted **Thermo Fisher Scientific Catalog #AB0600**

Thermo Scientific Autosampler Vial Kit **Thermo Fisher Scientific Catalog #03-060-016**

Eppendorf Snap-Cap Microcentrifuge Flex-Tube Tubes Amber **Fisher Scientific Catalog #05-402-31**

Hard-Shell 96-Well PCR Plates low profile thin wall skirted white/clear **BIO-RAD Catalog #HSP9601**

0.5 mm Zirconia/Silica Beads **Bio Spec Products Inc. Catalog #11079105z**

TissueLyser II	
Qiagen	**85300**

SAFETY WARNINGS

Chloroform is used in this protocol so please follow the appropriate safety guidelines for handling and disposing of halogenated solvents at your institution and use a fume hood for steps involving chloroform.

Wear proper PPE (gloves, safety goggle, and lab coat) for safety and to minimize contamination of samples. 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 according to your institution's protocols.

BEFORE STARTING

This protocol consists of steps for:
- Protein extraction from yeast cells
- Protein quantification
- Tryptic digestion

For this protocol you will need:
- an Eppendorf 5810R centrifuge with S-4-104 rotor or similar centrifuge
- a Qiagen TissueLyser II system
- a Molecular Devices Spectramax 250 microplate reader or similar plate reader
- an AB Sciex Veriti 96-well thermocycler or a similar incubator

Citation: Leanne Chan, Christopher Petzold (03/29/2021). Chloroform-Methanol Protein Extraction with Bead Beating for Yeast. https://dx.doi.org/10.17504/protocols.io.btj3nkqn

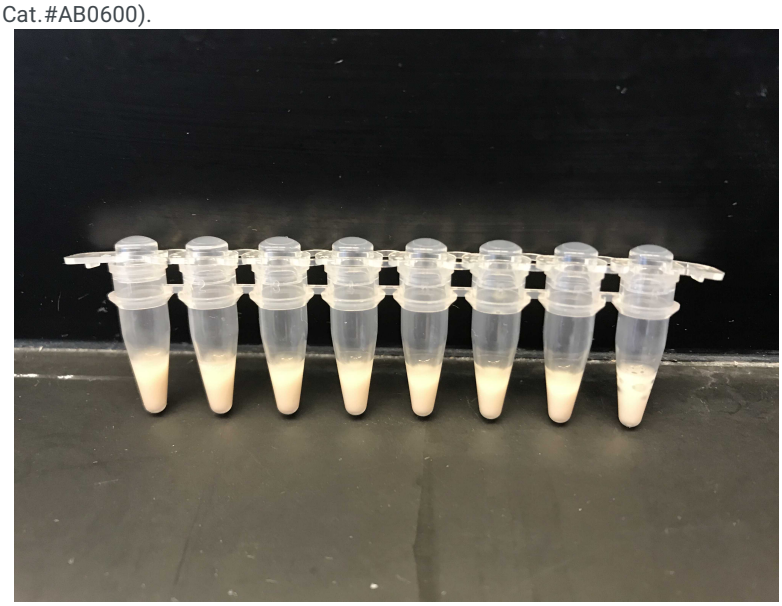
Bead-beating treatment 30m

1 Thaw cells at **Room temperature**

Note: If transferring directly from active cultures, omit this step. Adapt as needed for your specific organism and culturing conditions.

2

Transfer 2-4 OD*mLs of cells to 8-Strip PCR tubes (Axygen, Cat.#14-222-251) or a 96-well PCR plate (ThermoFisher, Cat.#AB0600).



A strip of PCR tubes filled 30-40% full of cell pellet is approximately 10-20 OD*mLs of cells.

3

Centrifuge at **4000 rpm, 25°C, 00:01:00** then remove the supernatent. **0 µl**

0 rpm

4 Add **60 µl** of LC-MS grade Methanol (VWR Scientific, Cat.#BJLC230-2.5). Pipet to resuspend well.

Note: You can combine step #4 and step #5 by adding 120 µl 1:1 methanol:chloroform together.

5 Add **60 µl** of Chloroform (Sigma-Aldrich, Cat.#34854). Pipet/vortex to mix.

Citation: Leanne Chan, Christopher Petzold (03/29/2021). Chloroform-Methanol Protein Extraction with Bead Beating for Yeast. https://dx.doi.org/10.17504/protocols.io.btj3nkqn

Use a fume hood when handling and pipetting chloroform.

6 

Add **50 µl** of beads (BioSpec 0.5mm Zirconia/Silica) to each PCR tube.

Note: You can also transfer resuspended cells into a new set of PCR tubes that is pre-loaded with beads.

7 Bead beat for 5 cycles of 1 minute at 30 Hz (max. frequency) in TissueLyser with 30 seconds on ice in between cycles.

8 Gently spin samples to bring all liquid to the bottom of the tube

Important: Spin before adding water/inducing phase separation.

Protein extraction 30m

9 Transfer supernatant (as much as possible) into new PCR tubes.

10 

Add **50 µl** of LC-MS grade Water (VWR Scientific, Cat.#BJLC365-2.5). Pipet/vortex to mix.

Note: This makes the supernatant now roughly 1:1:1 methanol:chloroform:water, if we assume ~20 µl lost within the beads; about 150 µl total volume remains in the PCR tubes.

11 

Centrifuge at **4000 rpm, 25°C, 00:01:00** .

12 

Carefully remove the top layer of solvent (Methanol + Water) by pipetting.

Citation: Leanne Chan, Christopher Petzold (03/29/2021). Chloroform-Methanol Protein Extraction with Bead Beating for Yeast. https://dx.doi.org/10.17504/protocols.io.btj3nkqn

13

Add **100 µl** of Methanol.

Tip: Break up the protein pellet by piercing it with the pipet tip, and add Methanol to the bottom of the tube or plate.

14

Centrifuge at **4000 rpm, 25°C, 00:02:00** .

15

Carefully discard solvent (Methanol + Chloroform).

Discard in an appropriate waste container for halogenated solvents.

16 Air-dry for **00:05:00** . 5m

Tip: Do not dry for longer than **00:45:00** or the pellet will be difficult to resuspend.

Dry samples in a fume hood.

17

Resuspend with **60 µl** **100 Milimolar (mM) Ammonium bicarbonate in 20% Methanol** .

Note: Samples are typically cloudy in this step. After trypsin digestion they will be nearly clear.

18

Store at **-20 °C** until ready for Protein Quantitation Assay.

Citation: Leanne Chan, Christopher Petzold (03/29/2021). Chloroform-Methanol Protein Extraction with Bead Beating for Yeast. https://dx.doi.org/10.17504/protocols.io.btj3nkqn

Protein Quantitation Assay (Lowry Method) 30m

19

Dilute samples 10 fold by adding **5 µl Protein sample, mix well right before transfer** to **45 µl Water** in 8-Strip PCR tubes or 96-well plate.

Note: The protein concentration can be determined by using several methods that are available in kits. We use the Bio-Rad DC Protein Assay (Bio-rad Laboratories, Cat.#500-0113, Cat.#500-0114) but the Bradford protein quantification assay is also commonly used. The accuracy of most protein concentration measurements can be variable, thus it is important to minimize differences in sample handling and to use replicates when quantifying the amount of protein in a sample.

20

Transfer 2 replicates of each of the following to Corning 96-Well Black Polystyrene Microplate (Fisher Scientific, Cat.#07-200-590):

5 µl Water (Blank)

5 µl Pierce Bovine Serum Albumin Standard Pre-Diluted Set (Std) (ThermoFisher, Cat.#23208)

5 µl Diluted samples, mix well right before adding to plate (Example 1-20)

Blank	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7				
Blank	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7				
1	2	3	4	5	6	7	8	9	10	11	12
1	2	3	4	5	6	7	8	9	10	11	12
13	14	15	16	17	18	19	20				
13	14	15	16	17	18	19	20				

Example Plate with 20 samples

21

Add **25 µl Bio-Rad DC Protein Assay Reagent A (Bio-rad Laboratories, Cat.#500-0113)** and wait **00:05:00** .

.

22

Add **200 µl Bio-Rad DC Protein Assay Reagent B (Bio-rad Laboratories, Cat.#500-0114)** and wait **00:10:00** .

.

Citation: Leanne Chan, Christopher Petzold (03/29/2021). Chloroform-Methanol Protein Extraction with Bead Beating for Yeast. https://dx.doi.org/10.17504/protocols.io.btj3nkqn

23

Read plate in the microplate reader (280 nm) and calculate protein concentrations.

Trypsin Digestion (5h - 16h) 5h

24 15m

Chemicals to prepare:

• **Prepare [M]100 Milimolar (mM) Tris(2-carboxyethyl)phosphine (TCEP) solution** by dissolving **28.7 mg TCEP** in **1 mL 100mM Ammonium Bicarbonate**

• **Prepare [M]200 Milimolar (mM) Iodoacetamide (IAA) solution** by dissolving **36.8 mg Iodoacetamide** in **1 mL 100mM Ammonium Bicarbonate**

•**Prepare [M]1 mg/ml Trypsin** by adding **1 mL 1mM HCl** to **1 mg Trypsin**

Store TCEP, IAA, and Trypsin in -20C.

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

25

Dilute protein samples to **[M]2.4 µg/µl** in **[M]100 Milimolar (mM) Ammonium Bicarbonate (AMBIC)**

Mix protein well before you dilute it.

26

Mix protein with TCEP, IAA, and trypsin in **[M]100 Milimolar (mM) Ammonium Bicarbonate (AMBIC)** .

The final concentrations will be **[M]2 µg/µl protein (in 50 ul total volume)** , **[M]5 Milimolar (mM) TCEP** , **[M]10 Milimolar (mM) IAA** , and **2 µl Trypsin (1 mg/ml)** (1:50 trypsin:protein ratio). Adjust as needed for your data acquisition protocols.

add **41.67 µl protein (2.4 ug/ul)**

add **2.5 µl TCEP (100 mM)**

add **2.5 µl IAA (200mM)**

Citation: Leanne Chan, Christopher Petzold (03/29/2021). Chloroform-Methanol Protein Extraction with Bead Beating for Yeast. https://dx.doi.org/10.17504/protocols.io.btj3nkqn

add **2 µl Trypsin (1 mg/ml)**

add **1.33 µl AMBIC**

up to **50 µl total volume**

If you do not have enough protein, you can set your final protein concentration to 1 ug/ul.

add **20.83 µl protein (2.4 ug/ul)**

add **2.5 µl TCEP (100 mM)**

add **2.5 µl IAA (200 mM)**

add **1 µl Trypsin (1 mg/ml)**

add **23.17 µl AMBIC**

up to **50 µl total volume**

27 

4h

Incubate at **37 °C** for **04:00:00** - **16:00:00** .

28 

Centrifuge at **4000 rpm, 4°C, 00:15:00** .

29 

Carefully pipet out clear liquid sample into plastic autosampler vials (ThermoFisherScientific, Cat.#03-060-016) or a 96-well plate (BIO-RAD, Cat.#HSP9601).

30 

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

Citation: Leanne Chan, Christopher Petzold (03/29/2021). Chloroform-Methanol Protein Extraction with Bead Beating for Yeast. https://dx.doi.org/10.17504/protocols.io.btj3nkqn