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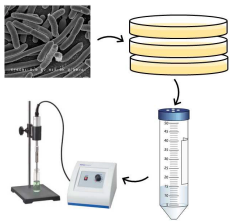
Generation of E. coli MG1655 whole cell lysate for proteomics applications



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Protocol status: Working
We use this protocol and it's working. It has been used to generate samples that have contributed to publications.

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Disclaimer

Abstract

This protocol describes the culture of *E. coli* MG1655 from a glycerol stock solution and subsequent lysis. It is intended for generating small quantities of whole cell lysate from cells grown in nutrient-rich (*e.g.* LB or TB medium) and/or nutrient-poor (*e.g.* M9 minimal) media for proteomics analysis. Culture and lysis of cells in a single Bio-Reaction tube minimizes the possibility of contamination.

All cell culture steps should be carried out using sterile materials and proper aseptic technique. This procedure is intended to be carried out in a biosafety cabinet, but it can also easily be performed on a bench top with a Bunsen burner.

Applications of this protocol at the National MagLab have utilized *E. coli* stocks from the Coli Genetic Stock Center at Yale University. The final lysate typically contains 300 - 500 µg/mL of protein for M9 minimal medium cultures and 750 - 1000 µg/mL for LB medium cultures, as determined by BCA assay.

The cell lysis protocol is adapted from **Preparing human WCL for analysis by qualitative TDMS** by Caroline DeHart, with a few changes.

Materials

Materials from specific manufacturers listed below are recommendations and can be replaced if needed. "MagLab equipment" refers to specific equipment in the MagLab ICR facility's BSL2-certified wet labs.

Consumables

- *E. coli* MG1655 culture (glycerol stock or similar)
- Nutrient agar plates
- Sterile inoculating loops
- Liquid nutrient medium
- Culture tubes, 16 x 150 mm
- 50 mL VWR Bio-Reaction tubes (cat. no. 76211)
- Tris base
- Sodium chloride
- N-lauroylsarcosine
- Thermo-Fisher Halt protease inhibitor cocktail or similar EDTA-free protease inhibitor cocktail
- HPLC-grade water
- Magnesium chloride
- Millipore Benzonase nuclease HC or New England Biolabs DNase I (cat. no. M0303S)

Generic equipment

- Heated orbital shaker
- Refrigerated centrifuge and rotor capable of 12,500 G with 50 mL tubes
- UV-vis spectrophotometer

MagLab equipment

- Thermo Scientific MaxQ 4000 heated orbital shaker
- Thermo Scientific Multifuge X3R centrifuge or Thermo Scientific Sorvall Legend XFR centrifuge with F15-8x50cy rotor
- Thermo BioMate 3 UV-vis spectrophotometer

Safety warnings

The practitioner is responsible for the safe handling, usage, and disposal of all hazardous materials associated with this protocol, including both chemical and biological hazards.

Cell culture

2d

1 Streak *E. coli* sample(s) on non-selective LB and/or M9 minimal media agar plates. Incubate at 37 °C until colonies are visible and well separated.

Note

LB medium plates will require 12 - 16 hours and M9 minimal medium plates will require 16 - 24 hours of growth.

To avoid overgrowth try incubating plates at 37 °C for 4 - 5 hours to kickstart cell growth, then allow them to finish growing in a room-temperature biosafety cabinet (or other sterile space) overnight.

2 Add 5 mL of liquid media to sterilized 16 x 150 mm culture tubes.

Note

Prepare two culture tubes per growth condition/medium type.

Typical glass culture tubes or 15 mL Bio-Reaction tubes can be used for this step.

3 Inoculate each culture tube with *E. coli* cells taken from a single colony on each agar plate.

Note

Cells used for inoculation should be grown on the same type of medium, *e.g.* use M9/agar plated cells to inoculate culture tubes with liquid M9 minimal medium.

4 Incubate culture tubes in an orbital shaker (250 rpm, 37°C) overnight (approximately 16:00:00). 16h

Note

Medium should be cloudy and completely saturated with bacterial cells.

5 Add 15 mL of sterile liquid media to 50 mL Bio-Reaction tubes.

Note

The final lysate typically contains 300 - 500 µg/mL of protein for M9 minimal medium cultures and 750 - 1000 µg/mL for LB medium cultures. Adjust the number of tubes according to the desired amount of total protein.

6 Inoculate each Bio-Reaction tube with 50 µL (1/300th volume) of the saturated culture grown overnight.

Note

Again, cells used for inoculation should be grown in the same type of medium, *e.g.* use M9 minimal medium overnight cultures to inoculate Bio-Reaction tubes with M9 minimal medium.

If desired, weigh 50 mL Bio-Reaction centrifuge tubes before adding media and write tare weight on each tube. This will make it easier to determine cell pellet weight later.

If smaller culture volume is desired, use 15 mL Bio-Reaction tubes with 5.0 mL of medium and inoculate with 17 µL of overnight culture.

7 Incubate tubes in an orbital shaker (250 rpm, 37°C). Use UV-vis spectrophotometer to monitor optical density at 600 nm (OD_{600}) and plot growth curve.

Note

Checking the OD uses up some of the culture. If desired, take the culture volume for this purpose from an extra tube which will be discarded and not used for lysis.

8 Grow cells to mid-to-late exponential stage, with OD_{600} of approximately 1.0.

Note

For previous applications of this protocol, *E. coli* MG1655 grew to OD_{600} ~ 1.0 in approximately 3.5 hours for LB medium and 10 hours for M9 minimal medium. Growth times vary, so it is strongly recommended to monitor the growth curve.

9 Centrifuge tubes at 4000 x g, 4°C, 00:15:00 , then decant culture medium from each tube leaving pellet behind. 15m

Note

Decanted medium is hazardous biological waste, deactivate by autoclave or bleach before disposal.

6,000 RPM yields RCF of 4,000 G for F15-8x50cy rotor.

10 Seal lids of Bio-Reaction tubes with Parafilm (or swap for lids without pores) and freeze cell pellets at -80 °C until ready to perform lysis (or at least one hour).

Cell lysis

4h

11 Begin cooling the centrifuge to 4 °C.

12 Prepare fresh lysis buffer according to the following table and chill on ice. This recipe produces 10 mL of lysis buffer, which is enough for cells from 45 mL of suspension culture. Scale up or down as needed.

	Component	Volume (mL)	Stock Concentration	Final Concentration
	Tris base, pH 7.5	0.2	1 M	20 mM
	Sodium chloride	0.2	5 M	100 mM
	N-lauroylsarcosine	1	10% w/v	1 % w/v
	HALT inhibitor cocktail	0.1	100X	1X
	HPLC-grade water	8.5		

Recipe for 10 mL of lysis buffer

Note

If using a different protease inhibitor cocktail, consult the manufacturer's instructions. For example, Thermo Scientific Pierce Protease Inhibitor Mini Tablets treat 10 mL of buffer per tablet.

The use of an EDTA-free protease inhibitor cocktail is recommended if using a nuclease in step 16, as EDTA will chelate Mg^{2+} ions needed for nuclease activity. If using EDTA, double the amount of $MgCl_2$ added in step 11.

13 Thaw cell pellets on ice for 15 mins, then resuspend each pellet in chilled lysis buffer. Use 1 mL of lysis buffer per 5 mL of suspension culture.

14 Incubate lysate on ice for 20 mins. Swirl to mix every 5 min.

15 Add 1 µL of 1 M $MgCl_2$ per 1 mL of lysis buffer to the lysate, to a final concentration of 1 mM.

16 Add nuclease to digest genomic DNA.

If using Benzonase nuclease HC:

Add 0.5 µL (125 U) of Benzonase nuclease HC for each mL of lysate. Pipette to ensure complete mixing.

If using DNAse I:

Add 10 µL (20 U) of New England Biolabs DNase I for each mL of lysate. Pipette to ensure complete mixing.

If using sonication:

It is acceptable to rely on a subsequent sonication step to break down genomic DNA and skip this step. It may be necessary to increase the number of sonication cycles to reduce lysate viscosity.

16.1 Incubate lysate at 37 °C for 20 min. Invert to mix every 5 mins.

16.2 Chill lysates on ice for 5 mins.

17 **Optional sonication step**

Place tube containing cell pellet resuspended in lysis buffer on ice. Set sonicator power to lowest setting and place probe tip close to the bottom of tube without touching. Turn on sonicator and adjust power until the surface of the buffer is agitated, but not foaming. Pulse for five cycles of 10 seconds followed by 30 seconds of rest.

18 Pellet cellular debris by centrifugation at 12,500 G for 20 mins at 4 °C.

Note

Max RCF for conical bottom tubes is typically 12,500 G, but much lower for freestanding tubes!

10,400 RPM yields RCF of 12,576G at R_{max} for F15-8x50cy rotor.

19 Determine total protein concentration for each lysate by performing a Bicinchoninic acid (BCA) or other appropriate assay.

20 Aliquot lysates into smaller portions appropriate to downstream applications (*e.g.* 50 or 100 µg for proteomics analysis). Store aliquots at -80 ºC until ready to use.

Note

Aliquots of lysate should only be thawed one time to minimize protein degradation.