

Standard Operating Procedure for Hazardous Chemicals

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Building and rooms: Davison Life Sciences Building, Lab B310

Chemical(s)	Magnesium chloride (<u>MgCl₂</u>), polyethylene glycol 3350 (<u>PEG</u>), dimethylsulfoxide (<u>DMSO</u>), liquid nitrogen (<u>LN₂</u>).
Process	Preparation of <i>E. coli</i> Competent Cells
Specific Hazards <i>referred to MSDSs for more detailed information</i>	<u>MgCl₂</u> and <u>PEG</u> are slightly hazardous as skin/eye/respiratory irritants and as skin permeators. <u>PEG</u> is also combustible. <u>LN₂</u> is a simple asphyxiant and can cause cryogenic burns. <u>DMSO</u> easily penetrates skin and may allow enhanced skin absorption of a number of other chemicals. Inhalation, ingestion, or absorption may cause dizziness, nausea, and headache. <u>DMSO</u> is also combustible.
Personal protective equipment	Must wear 3-5 mil nitrile gloves; cryogloves should be used for pouring large volumes of <u>LN₂</u> . Chemical safety goggles and lab coat should be worn when splash potential exists.
Engineering/ventilation controls	Use adequate ventilation to keep airborne concentrations of <u>DMSO</u> low.
Special handling procedures and storage requirements	Store <u>MgCl₂</u> on bench top away from oxidizing agents. Keep <u>PEG</u> away from heat, oxidizing agents, and sources of ignition; store with tightly closed lid. Store minimal volumes of <u>LN₂</u> in covered Styrofoam containers for procedural use. Keep <u>DMSO</u> tightly closed and away from ignition sources, heat, strong oxidizing agents, strong acids, strong bases, and acid chlorides.
Spill and accident procedures <i>for hazardous chemicals only</i>	<p><u>Skin exposure:</u> Rinse affected skin with plenty of water while removing contaminated clothing/shoes. Rinse for > 15 minutes.</p> <p><u>Eye exposure:</u> Wash eyes for > 15 minutes.</p> <p><u>Inhalation:</u> Remove to fresh air immediately. If not breathing give artificial respiration (NOT mouth-to-mouth).</p> <p><u>Ingestion:</u> Do not induce vomiting.</p> <p>For all cases, seek medical attention immediately.</p>
	<u>Small</u> (< 2L): Vacuum or sweep up material and place into a suitable disposal container, avoiding generation of dust. Notify PIs.
	<u>Large</u> (> 2L): Evacuate the room, notify PIs and call 2-5801 to request emergency spill assistance from the Environmental Safety Division.
Waste disposal	<u>DMSO</u> in high concentrations should be collected as hazardous waste according to the SOP for Hazardous Waste Disposal.
Special approval	No special authorization needed after SOP training & reading MSDSs.
Reviewed by	Name/date:

Preparation of *E. coli* Competent Cells

This method is effective for preparing *Escherichia coli* to be used for genetic transformation.

Solutions and Reagents needed

Luria-Bertani broth (LB)

1X Transformation and Storage Solution (TSS), store at 4°C

LB 9 mL

PEG 3350 [10% (w/v)] 1.0 g

Magnesium chloride (50 mM) 47.6 mg

Adjust pH to 6.5

Filter sterilize using sterile 0.45 µm cellulose nitrate filter

DMSO [5% (v/v) final concentration] 1.0 mL, added in laminar flow hood

Alternative preparation: Combine PEG, MgCl₂, and DMSO in premade LB, adjust pH to 6.5, then filter sterilize through a **nylon** syringe filter.

Liquid nitrogen

Precautions: Keeping cells cool throughout steps 4-9 is critical to the transformation efficiency of your cells. Utilize standard sterile technique procedures when working with bacteria to avoid contamination.

Procedure

1. Streak an LB plate with the desired strain of *E. coli* (use selection antibiotic as appropriate) and grow overnight at 37°C.
2. Prepare a 5 ml tube of LB and add a single colony from the plate. Grow overnight at 37°C on a rotating shaker.
3. Prepare a 125 ml Erlenmeyer flask of 50 ml LB and add 500 µl of culture from the tube. Grow to the early exponential phase (OD₆₀₀ 0.3-0.4), about 3 hrs, at 37°C on a rotating shaker.
4. Chill flask in ice water (0°C) for 30 min.
5. Transfer 45 mL of culture to 50 mL Falcon centrifuge tubes held in ice water, then centrifuge the tubes for 10 min at 1000 g at 4°C.
6. Remove the supernatant and gently resuspend the pellet in 5.0 mL **ice cold** 1X TSS, holding the tube in ice water as you work.
7. Aliquot 100 µl each to 50 sterile and prechilled microcentrifuge tubes.
8. At this point you may proceed directly to transformation or snap freeze the tubes in liquid nitrogen and store at -80°C.

Heat Shock Transformation: (Note: It is often useful to run a control transformation to determine transformation efficiency before using a batch of cells for experimental transformations)

9. Add 1-5 ng plasmid DNA to 1 tube of competent cells. Hold on ice for 15-30 min if using frozen cells, or 5 min if using freshly prepared cells.
10. Heat shock in 42°C water bath for 2 min, then return to ice for ~5 minutes.
11. Add 0.5-1.0 ml of LB(+glucose) to the tube, then incubate on a 37°C rotating shaker for 30 min to 3 hrs.
12. Plate out 50-200 ul of culture on an LB plate containing an appropriate selection agent. Remaining culture may be held at 4°C for up to 14 days in case you wish to plate out more later.
13. Grow up plates overnight at 37°C and proceed to colony PCR to confirm transformation.

Mini-scale: Use 1 ml (from 5-ml culture) and resuspend in 100 ul TSS.

References:

Chung, C.-T., Niemela, S.L., and Miller, R.H. 1989. One-step preparation of competent *Escherichia coli*: transformation and storage of bacterial cells in the same solution. PNAS 86: 2172-2175.

Chung, C.-T., and Miller, R.H. 1993. Preparation and storage of competent *Escherichia coli* cells. Methods in Enzymology 218: 621-627.