MDH Protocol:

Instructions - Bacterial Culture for MDH Expression

Bacterial Cultures: There are typically two reasons for culturing bacteria in our lab. First is to purify DNA and the other to express protein. The volume of culture depends on the plasmid purification method and the final yield you wish to achieve. Please refer to any manufacture's protocols for specifics. Below is a GENERAL guideline that can change depending on the volume of culture you need.

Antibiotic	**Stock Concentration**	**Storage**	**Working Conc (dilution)**
Ampicillin (Sodium Salt)	50 mg/ml in water (500X)	-20°C	100µg/ml (2 µl of stock/ml)
Chloramphenicol	34 mg/ml in EtOH (200X)	-20°C	170 µg/ml (5 µl of stock/ml)
Kanamycin	25 mg/ml in water (500x)	-20°C	50 µg/ml (2 µl of stock/ml)
Streptomycin	10 mg/ml in water (200X)	-20°C	50 µg/ml (5 µl of stock/ml)
Tetracycline HCl	5 mg/ml in EtOH (100X)	-20°C	50 µg/ml (10 µl of stock/ml)

To maintain cells that only carry your plasmid, an antibiotic should be included in all phases. Ampicillin acts to damage the membranes of *E. coli* by inhibiting the crosslinking of the bacterial membrane. Another commonly used antibiotic is kanamycin. This drug works by blocking protein synthesis at the mRNA level. It is important to remember that the antibiotic will "break down" (usually a hydrolysis of the compound) above 60°C or if left at room temp for several days. We typically keep concentrated antibiotic in the freezer. While it is not proper to re-freeze, we find little problem re-freezing unused antibiotic.

Culturing Cells – Starting your culture should almost always be done from an isolated colony from a freshly streaked plate. In a pinch you can use from glycerol stock but you can lose your plasmid this way. Don't trust the plates that have been around for too long (a month or so). They may look good but are likely dead or contaminated with a mold or fungus or some other nasty critter. If the plates are old, either transform a new set of cells or chip of a bit of frozen glycerol stock from the top of the tube with a pipet tip (do NOT let the frozen cell thaw) and spread on an LB Agar plate with antibiotic. Culture overnight in the 37°C incubator and store the new plate wrapped in parafilm at 4°C.

Bacterial Strains –

- DH5alpha: Not appropriate for protein expression, good for plasmid purification or storage.
- BL21 – good basic cells for protein expression, is deficient (reduced) in proteases.
- XJ Autolysis *E. coli* strains are an alternative for bacterial transformation and lysis. These strains are efficiently lysed following arabinose-induced expression of the bacteriophage λ endolysin protein, coupled to a single freeze-thaw cycle.

  XJa Autolysis™ (E. coli, K-strain JM109) Grows quite well, especially when media is supplemented with 1 mM magnesium This strain lyses very easily . Suitable for general screening, but proteases may degrade small or otherwise unstable recombinant proteins. XJb Autolysis™ (E. coli, B-strain BL21) A very robust strain, reaching higher OD's than E. coli K-strains. XJb lysis efficiency is 10-20 % lower than XJa. For optimal lysis, more care needs to be taken when selecting the lysis buffer. However, even very low concentrations of a detergent improve lysis significantly XJb is ideal for recombinant protein expression.

  - Induce λ with 3 mM L-arabinose with 1 mM $MgCl_2$ ***when adding IPTG****. (make as a 500 or 1000X stock). Do not add arabinose until adding IPTG*

Antibiotic and Bacterial Strain Notes:

All MDH clones (EXCEPT wgMDH) are cloned into pET28 vectors. pET28 plasmids are **Kan resistant.** These plasmids are under the control of the strong bacteriophase T7 transcription. These constructs MUST be expressed in a bacteria that contain the T7 RNA Polymerase gene, gamma DE3 lysogen **(DE3).**

wgMDH and their mutants are in Qiagen's pQE60 vector **use AMP** for any wgMDH wild-type and mutant construct. pQE60 plasmids do not need the bacteria carrying DE3 for expression.

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Starting Volumes – LB Media with appropriate antibiotic

- 50 ml culture - use 1 ml of 10 ml starter culture
- 250 ml culture - use 5 ml of 10 ml starter culture
- 1 L culture - use 20 ml of starter culture.

1) Starter Culture - Transfer each colony the appropriate volume of media indicated above and incubate at 37°C (shaking) for 5-24 hours. *For low expressors, the density of culture should never exceed 0.5 AU until induction.*

- Use room temp LB with antibiotic to seed the media.
- From a freshly transformed cell transfer a colony (some will pick several colonies if only purifying proteins) and seed the starter culture.
    - *While not optimal, starter cultures can be seeded from a chunk of frozen glycerol stock. NEVER let the glycerol stock thaw.*
- Incubate the culture with shaking at 37°C until OD600 0.5-0.6. Do not allow the culture to go above 0.6 OD. If the bacteria grow too dense, the cells will inhibit the protein expression. The culture can be stored at 4°C overnight.
    - *Alternative to grow the starter overnight at a high density. Strong MDH expressors work well in these conditions.*
- Once the culture has reached the correct OD - Save one ml of culture, centrifuge down at max speed for 5 min. in a microfuge tube. Discard supernatant and freeze pellet at -20oC. This is the pre-induced (uninduced) sample for later analysis.
- Place the culture at 4°C (for overnight – no longer) or continue with the procedure (expansion of starter culture).

2) Expansion of Starter Culture-

- *Expand the culture by adding the starter culture larger volume LB with antibiotic (room temp) and incubate for 1-4 hours until culture density reaches 0.5-0.6 OD600. Top the flask with cotton or a vented culture flask cap to allow culture to oxygenate without getting environmental contamination. Do NOT seal with parafilm or aluminum foil.*

3) Protein Expression Induction. *Note: IF using XJa or XJb autolysis cells. You must add L-Arabinose/MnCl You must add L-Arabinose/$MgCl_2$ when adding IPTG. Add Autolysis protocol to your experiment if using this strain of bacteria.*

37°C INDUCTION

- *Induce expression after culture has reached OD 0.5-0.6 by adding IPTG to a final concentration of 1.0- 0.5 mM. IPTG is a can be stored as a frozen solution at-20oC. Refreeze unused IPTG.*
- *Incubate with shaking for 3 to 4 hours at 37°C.*

ROOM TEMP (20°C) INDUCTION.

- *Induce Expression (see note below) – After culture has reached OD 0.5-0.6, cool down to room temperature by placing in refrigerator or in an iced water bath. Then induce cells with 0.5 -1.0 mM IPTG. Refreeze unused IPTG.*
  Note – for difficult expression may need to induce at 16°C for 48 hours with 0.5 mM IPTG.

4) Collect Cells – *Centrifuge the cells at 3,500 x g for 20 min.*

- *- Resuspend cells in ICE COLD PBS and re-centrifuge in an appropriate sized tube.*
- *- Remove the supernatant and freeze pellet in falcon tube or plastic baggie for later processing.*

5) Cell Lysis - *Lyse cells using appropriate protocol (autolysis or other)*