Protocol for preparation of minimal media

1. Preparation of stock solutions

A. Vitamin Solution (1000 ml)

1.1 mg biotin	Store in the refrigerator
1.1 mg folic acid*	Store at room temperature
110 mg PABA (para-aminobenzoic acid)	Store in the refrigerator
110 mg riboflavin*	Store at room temperature
220 mg pantothenic acid	Store in the refrigerator
220 mg pyridoxine HCl*	Store at room temperature in dessicator
220 mg thiamine HCl*	Store at room temperature
220 mg niacinamide	Store at room temperature

* Note that these vitamins are light sensitive.

Add 500 ml H_2O and 500 ml high-purity ethanol to the vitamins and then filter sterilize. The solution will be bright yellow. Store the vitamin solution in the refrigerator in a dark bottle (we wrap our solution with aluminum foil).

Proper storage for each of the individual vitamins is shown to their right.

B. Metal Stock Solution (100 ml)

8 ml concentrated HCl
5 g $FeCl_2 \cdot 4H_2O$
184 mg $CaCl_2 \cdot 2H_2O$
64 mg H_3BO_3
40 mg $MnCl_2 \cdot 4H_2O$
18 mg $CoCl_2 \cdot 6H_2O$
4 mg $CuCl_2 \cdot 2H_2O$
340 mg $ZnCl_2$
605 mg $Na_2MoO_4 \cdot 2H_2O$

Bring to a volume of 100 ml with H_2O. Initially the solution will be green and you will need to stir for several hours before everything dissolves. Store the metal stock solution at room temperature.

C. "O" Solution (500 ml)

Add 10 ml Metal Stock Solution (above) to 26.8 g $MgCl_2 \cdot 6H_2O$ then add H_2O to 500 ml. Filter sterilize and store at room temperature.

Note that the Metal Stock Solution is only used to prepare the "O" solution and is not added to the minimal media.

D. "SBMX" Solution (500 ml)

To 16.5 g KH_2PO_4, 87.5 g K_2HPO_4, and 18.25 g NaCl add H_2O to 500 ml. Autoclave and store the "SBMX" solution at room temperature. The pH of this solution should be 7.5, however if done correctly no pH adjustment should be necessary.

E. "S" Solution (100 ml)

To 4.8 g K_2SO_4 add H_2O to 100 ml. Autoclave and store the "S" solution at room temperature.

F. Thiamine Solution (Small volume)

Make up a 1 mg/ml solution of thiamine in H_2O. Store the thiamine solution in the refrigerator. We generally make smaller stock solutions of the thiamine solution to avoid letting the solution get to old.

2. Preparation of 1 liter of minimal media

To create the growth media add together
940 ml H_2O
40 ml "SBMX" solution
1 ml "S" solution
Autoclave and cool

Mix together
2 ml "O" solution
1 ml vitamin solution
1 ml thiamine solution
Filter sterilize the mixture and add to cool growth media

Add together
1 g $^{15}NH_4Cl$ or $(^{15}NH_4)_2SO_4$
5 ml H_2O
Filter sterilize and add to cool growth media

Add together
4 g glucose
10 ml H_2O
Filter sterilize and add to cool growth media

Add antibiotic of choice

Note: We have found the most cost effective amount of glucose to add is between 3 and 4 grams.

3. <u>Cell Growth</u>

- A 5 ml culture of LB (10 g Bacto tryptone, 5 g Bacto yeast extract and 5 g NaCl per liter) is inoculated using a single colony from a fresh LB plate and grown for ~ 6 hours at 37º C with shaking; they should reach on OD_{600} of 0.5 - 1.0.
- Use 0.2 ml of the LB culture to inoculate a 25 ml minimal media culture and grow overnight at 30 - 37º C with shaking; they should grow to an OD_{600} of 2-3.
  - Note: Use 25 ml starter culture per liter. Therefore, if more than 1 liter of minimal media will be induced the starter culture size will need to be increased.
- Spin down the cells (~2000g) to remove supernatant and resuspend cells in 10 ml of minimal media.
  - Note: We often skip this spin down step and simply inoculate the 1 liter of minimal media culture with the 25 ml overnight starter culture.
- Use the 10 ml of the resuspended cells from the overnight starter culture to spike 1 liter of minimal media culture. Grow and induce cells as normal.

<u>Reference</u>: Weber DJ, Gittis AG, Mullen GP, Abeygunawardana C, Lattman EE and Mildvan AS. "NMR docking of a substrate into the X-ray structure of staphylococcal nuclease." *Proteins* (1992**)** **4**, 275-287.

<u>Special Notes</u>:

- The original Weber paper used two additional solutions. One is a T-U solution of thymine and uracil and the other is the "M" solution which contains MOPS buffer and tricine. These both have been dropped in the protocol we now use. I am not positive but the removal of the "M" buffer was likely done to remove a possible carbon source for the bacteria to use.

- Glucose can cause some repression of IPTG inducible transcription. Therefore, if you are inducing at low cell densities $OD_{600} < 0.8$ where significant glucose may be present a higher IPTG concentration may be needed.

- In general it is a good idea to try growing cultures to different OD_{600} values, inducing for various times and with various amounts of IPTG and to try expression at lower temperatures such as 30º C. All of these parameters can be adjusted to maximize protein expression and solubility.