

## Mamalian Cell Culture Media Preparation

DMEM high glucose, L15 and RPMI

- suppliments and antibiotic/mycotics are found at the end.



MSUM Biochemistry &  
Biotechnology

**DMEM High Glucose** - 4 mM L-glutamine 4500 mg glucose/L 1500 mg/L sodium bicarbonate

Optimized energy sources for protein production and nucleic acid metabolism while limiting toxic ammonia build-up To be used with 5% CO<sub>2</sub> to maintain pH. NOTE: This reduced level of sodium bicarbonate (NaHCO<sub>3</sub>, 1.5 g/L) is intended for use in 5% CO<sub>2</sub> in air. Additional sodium bicarbonate may be required for use in incubators containing higher percentages of CO<sub>2</sub>.

Imporant Note: ENSURE that the media has the correct anitbiotics or antimicotics for the cells you culture. Many human cells will not grow if Antimycotics are used. Some cells need an antibiotic selection agent. ALWAYS refer to the specifics of each cell line before using any medium.

If your cells require antimicotic, add 10 ml of 100X Antimycin A to each liter of medium. (**Label w/Antimycotic**)

1 liter of **DMEM Base Medium with Glutamine (Label as: DMEM Base w/Pen-Strep)**

- 1 bottle DMEM (sigma powder - fridge)
- 2.1g NaHCO<sub>3</sub> (Sodium Bicarbonate)
- 10ml of 100 x Glutamine (freezer)
- 10ml of 100 x Pen/Strep (freezer) \*
- \* Can use combination of Pen/Strep/Glutamine
- pH to 7.2
- Q.S. to 1 liter using box water

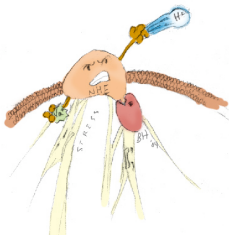
(Note: filter should be put on and taken off in cultured hood.)

1000 ml **Complete (Label as: DMEM Complete w/Pen-Strep)**

- 900 ml DMEM Glutamine Base
- 100 ml Serum (FBS in -80 freezer)
- Filter sterilize into autoclaved bottle

500 ml **Low Serum Medium - 0.5% Serum (Label as: DMEM Low Serum w/Pen-Strep)**

- 475mL DMEM Base Media
- 25mL Complete Media (10% RPMI Serum)



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### RPMI – 1640

Modified to contain 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 mg/L glucose, and 1500 mg/L sodium bicarbonate.

1 liter of **RPMI Base Medium (Label as: RPMI Complete Base w/Pen-Strep)**

- 1 bottle RPMI (liquid - fridge)
  - \* check to see that the liquid contains glutamine and bicarbonate
  - \* if not add 2.1g NaHCO<sub>3</sub> (Sodium Bicarbonate) and 10 ml Glutamate (100X)
- 10ml of 100 x Pen/Strep (freezer)

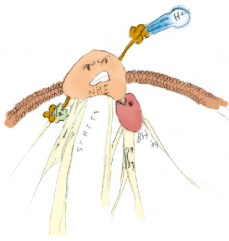
(Note: sterile transfer in culture hood with sterile pipette to avoid the need to filter.)

1000 ml **Complete (Label as: RPMI Complete w/Pen-Strep)**

- 900 ml RPMI Base w/Pen-Strep
- 100 ml Serum (FBS in -80 freezer)
- Filter sterilize into autoclaved bottle

500 ml **Low Serum Medium - 0.5% Serum (Label as: RPMI Low Serum w/Pen-Strep)**

- 475mL RPMI Base w/Pen-Strep
- 25mL Complete RPMI Medium
- Filter sterilize into autoclaved bottle



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**Leibovitz's L-15** Optimized energy source for protein production and nucleic acid metabolism while limiting toxic ammonia build-up. No added sodium bicarbonate. To be used without CO<sub>2</sub>. With 2 mM L-glutamine.

To Prepare 1 liter of **Base Media**

- 1 bottle L15 (Powder - fridge)
- 2.1g NaHCO<sub>3</sub> (Sodium Bicarbonate)
- pH to 7.2
- MEM Amino Acids (Freezer – see aa instructions)
- MEM Vitamins (Freezer – see instructions)
- 10ml Pen/Strep/Glut (freezer) \* or use both pen/strep and Glut.
- Q.S. to 1 liter using box water

To Prepare 400ml **Complete Media** (10% Serum)

- 360mL L15 Base Media
  - 40mL Fetal Bovine Serum (FBS in -80 freezer)
  - Filter sterilize into autoclaved bottle(s)
- (Note: filter should be put on and taken off in culture hood.)

To Prepare 500ml **Low Serum Media** (0.5% Serum)

- 475mL Base Media
  - 25mL Complete Media (10% L15 Serum)
  - Filter sterilize into autoclaved bottle
- (Note: filter should be put on and taken off in culture hood.)

## Minimum Essential Medium Eagle (MEM)

To Prepare 1 liter of **Base Media**

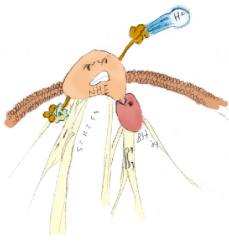
- 1 bottle MEM (fridge)
- 2.1g NaHCO<sub>3</sub> (Sodium Bicarbonate)
- 10 ml glutamine
- 0.11 g Na Pyruvate
- pH to 7.2
- Dilute to 1 liter using box water
- .01mm non essential amino acids – should be in media

## Ham's F-12

To Prepare 1 liter of **Base Media**

- 1 bottle Ham's F-12 (Research fridge)
- 10ml 40 mM Proline Stock (research fridge)
- 2.1 g NaHCO<sub>3</sub> (Sodium Bicarbonate)
- pH to 7.2
- Dilute to 1 liter using box water





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### **Penicillin Streptomycin Solution (100x)** 10,000 I.U./ml Penicillin 10,000 µg/ml Streptomycin ATCC Catalog No. 30-2300

10,000 I.U./ml Penicillin, 10,000 (ug/ml) Streptomycin For reducing the chances of microbial contamination in cell culture. Between 0.5 and 1 ml of Penicillin-Streptomycin solution are added to 100 ml of cell culture media for a final concentration of 50 to 100 I.U./ml penicillin and 50 to 100 (ug/ml) streptomycin. NOTE: Most cell culture technologists avoid using antibiotics and antimycotics for routine culture work. Antibiotics and antimycotics may mask contamination by susceptible bacteria and fungi while permitting mycoplasma to flourish unnoticed. Antibiotics and antimycotics may interfere with the metabolism of sensitive cells in culture. However, one may elect to introduce antibiotics and antimycotics for short periods to primary cultures or as a safeguard while propagating specific valuable stocks.

### **Amphotericin B Solution, 250 µg/ml (100X)** Catalog No. [30-2301](#)

For reducing the chances of fungal or yeast contamination in cell culture. One ml of Amphotericin B solution is added per 100 ml of cell culture media for a final concentration of 2.5 (ug/ml) Amphotericin B can be added to the water of cell culture incubators at 1% to control fungal growth. Most cell culture technologists avoid using antibiotics and antimycotics for routine culture work. Antibiotics and antimycotics may mask contamination by susceptible bacteria and fungi while permitting mycoplasma to flourish unnoticed. However, one may elect to introduce antibiotics and antimycotics for short periods to primary cultures or as a safeguard while propagating specific valuable stocks. CAUTION: the use of antimycotics (as Amphotericin B) at high concentrations and/or for long periods of time can be toxic to some cell lines.

### **L-Glutamine Solution, 200 mM**

Added as a supplement to cell culture media. L-Glutamine is an essential amino acid required by virtually all mammalian and insect cells grown in culture. L-Glutamine is also more labile in cell culture solutions than other amino acids. The rate and extent of L-glutamine degradation are related to storage temperatures, age of the product, and pH. Catalog No. [30-2214](#)

### **G418 Sulfate, Powder**

Catalog No. [30-2305](#)

Used as a dominant selective agent in cell transfection experiments.

### **Trypsin EDTA Solution** 0.25% trypsin, 0.53mM EDTA in Hanks' Balanced Salt Solution (HBSS), modified without calcium chloride or magnesium chloride

0.25% Trypsin/0.53 mM EDTA in Hanks Balanced Salt Solution without calcium or magnesium. For dissociation of cell monolayers. Catalog No. [30-2101](#)

