REAGENTS FOR PREPARATION OF TISSUE CULTURE MEDIA

**IMDM** = Iscove’s Modified Dulbecco’s Medium
   GibcoBRL cat # 12200-036 (powder)

**MEM Eagle** = Minimum Essential Medium Eagle (Fish cells)
   Sigma cat # M-4655

**MEM Eagle** = Minimum Essential Medium Eagle (FHM cells)
   Dissolve whole bottle in 1L of distilled H₂O plus 0.35 g NaHCO₃
   Adjust pH to 7.0 using 1N NaOH
   Sigma cat # M-1018 (powder)

**NEAA** = MEM Non-Essential Amino Acids Solution (10 mM = 100x)
   GibcoBRL cat # 12383-014

**PS** = Penicillin-Streptomycin (10,000 units/ml)
   GibcoBRL cat # 15140-122
   Aliquot 10 ml/tube and store at -20°C

**Insulin** = Insulin from Bovine Pancreas
   Sigma I-6634 1 gram vial (powder) MW = 5733.3
   Prepare at 5 mg/ml in water (1 gram vial in 200 ml)
   Aliquot 10 ml/tube and store at -20°C

**2-Me** = 2-Mercaptoethanol (or β-Mercaptoethanol) - (55mM = 1000x)
   GibcoBRL cat # 21985-023

**Primatone** - Enzymatic digest of animal tissue
   Sheffield Products Division (powder)
   Prepare at 10% in water
   Store at -20°C for long term or 4°C for short term

**Sodium Bicarbonate** = NaHCO₃
   Sigma cat # S-6014 (powder) MW = 84.01

**Kanamycin** = Kanamycin sulfate solution (10 mg/ml)
   Sigma K-0129

**L-Glutamine** (100x liquid)
   GibcoBRL cat # 25030-081

**A6 Sup** = Supernatant collected from A6 cell line
   Collected, centrifuged and stored at 4°C
FBS = Fetal Bovine Serum  
Atlanta Biologicals cat # S11150  
Aliquoted 10 ml/tube and stored at -20°C

XS = Xenopus Serum  
Collected from frogs and stored at -20°C  
Spin in centrifuge before use to remove debris

TISSUE CULTURE MEDIUM

**Mammalian Serum Free Basic Medium** (MSF)  
Choose from the following depending on the amount of media to be made

<table>
<thead>
<tr>
<th></th>
<th>1 Liter</th>
<th>2 Liters</th>
<th>3 Liters</th>
<th>4 Liters</th>
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</thead>
<tbody>
<tr>
<td>IMDM</td>
<td>1 pkg</td>
<td>2 pkg</td>
<td>3 pkgs</td>
<td>4 pkgs</td>
</tr>
<tr>
<td>PS</td>
<td>10 ml</td>
<td>20 ml</td>
<td>30 ml</td>
<td>40 ml</td>
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<tr>
<td>NEAA</td>
<td>10 ml</td>
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<td>30 ml</td>
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<tr>
<td>Insulin</td>
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<tr>
<td>2-Me</td>
<td>1 ml</td>
<td>2 ml</td>
<td>3 ml</td>
<td>4 ml</td>
</tr>
<tr>
<td>Primatone</td>
<td>3 ml</td>
<td>6 ml</td>
<td>9 ml</td>
<td>12 ml</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>3.02g</td>
<td>6.04g</td>
<td>9.06g</td>
<td>12.08</td>
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</tbody>
</table>

Mix all ingredients accordingly  
Adjust to the appropriate volume with 3x glass distilled water  
pH to 7.0 with 10N NaOH  
Filter thru 0.2µm filter and store at 4°C

THE FOLLOWING ARE PREPARED USING THE MSF MEDIUM ABOVE

**Amphibian SF Medium** (ASF) / (ASF + 10% FBS)  
Used for the A6 and DWJ cell lines

- 200 ml MSF medium  
- 60 ml 3x distilled water  
- 5 ml FBS / (25 ml FBS)  
- 5 ml Pen-Strep  
- 500 µl Kanamycin

Filter thru 0.2µm filter and store at 4°C
**Amphibian SF Medium + A6 Supernatant** (ASF + A6)
Used for the B3B7 cell line

400 ml MSF medium  
120 ml 3x distilled water  
40 ml A6 sup  
10 ml FBS  
10 ml Pen-Strep  
1 ml Kanamycin

Filter thru 0.2um filter and store at 4°C

**Amphibian SF Medium + A6 Supernatant + Xenopus Serum** (ASF + A6 + XS)
Used for the 15/0, 15/40, and ff-2 cell lines

200 ml ASF + A6 medium  
500 µl XS (0.25%)  
5 ml Pen-Strep  
500 µl Kanamycin

Filter thru 0.2um filter and store at 4°C

**Amphibian SF with no Goodies** (for wash solution)
Used for washing antibody coated plates before plating cells

200 ml MSF medium  
60 ml 3x distilled water

Filter thru 0.2um filter and store at 4°C

**Fish Medium**
Used for EPC cells

200 ml MEM Eagle (M-4655)  
10 ml FBS  
5 ml Pen-Strep  
500µl Kanamycin

Filter thru 0.2um filter and store at 4°C
**FHM Medium**

Used for FHM cells

- 200 ml MEM Eagle (M-1018)
- 8 ml FBS
- 5 ml Pen-Strep
- 500 µl Kanamycin

Filter thru 0.2 um filter and store at 4°C

**OTHER REAGENTS FOR TISSUE CULTURE**

**BSA** = Bovine Serum Albumin
Sigma A-4503 100 grams

**EDTA** = Ethylenediaminetetraacetic Acid (Disodium, Dihydrate MW 372.2)
Sigma E-5134

**Amphibian Phosphate Buffered Saline (APBS)**

For 20 Liters

- Sodium Chloride (NaCl) 6.6 g/L  132 grams
- Sodium Phosphate (Na₂HPO₄) 1.15 g/L  23 grams
- Potassium Phosphate (KH₂PO₄) 0.2 g/L  4 grams

pH to 7.7 with 10N NaOH
Filter thru 0.2um filter as needed for tissue culture work

**APBS + 1% BSA**

Used as a blocking solution
Add 10 grams BSA to 700mls of APBS
Stir well until dissolved
Adjust volume to 1 Liter by adding additional APBS
Filter thru 0.2um filter and store at 4°C
**APBS + 0.1% EDTA**

Used to remove adherant cells
Add 0.3 grams EDTA to 250 mls of APBS
Stir well until dissolved (may need to heat slightly to get into solution)
Adjust volume to 300 mls by adding additional APBS
Filter thru 0.2um filter and store at 4°C

**RPMI 1640 Medium** (For 1 Liter)

1 package of powder (Gibco cat #
10mls PS
1ml 2-Me
2g’s NaHCO₃

pH to 7.0 with HCL
Filter thru 0.2um filter and store at 4°C
FREEZING and THAWING CELLS

PREPARE FREEZING MEDIUM:
- 50% Medium (appropriate for cell line being frozen)
- 50% FBS
  To this add 10% Hybridmax DMSO (Sigma cat # D-2650)
A batch can be made and aliquots can be frozen at -20°C

FREEZING:
1. Count cells to be frozen
2. Spin and resuspend at 1x10^6 to 5x10^6/ml in cold freezing medium
3. Aliquot 1ml/freezing vial - keep on ice
4. Cells can be frozen in the nitrogen storage facility (ATRF)
   You need to contact Colleen x5-1778 to set up an appointment in advance
   You also need to have a control vial = freezing medium only
5. Colleen will place the vials in liquid N₂ when the freezing is complete
6. Be sure to map out what cell line was frozen and where it was placed

THAWING:
1. Remove vial from liquid N₂ and place it on ice (call ahead)
2. Immediately when returning to the lab suspend the vial in water to thaw
   **NOTE:** It is very important that the vial only be suspended until it starts to thaw. Watch it carefully, as soon as the ice starts to melt remove it and thaw the rest of the way in your hand!
3. Dilute the vial as desired in pre-warmed medium (10% FBS) and place it at appropriate temperature as soon as possible.
   **Example:**
   Normally for a 1ml vial frozen at 5 x 10⁶/ml I thaw as follows:
   1. In a six well plate set up three wells with different amounts of cells
      1ul, 2ul and 10uls of cells in a total volume of approximately 5 ml
   2. In a small - medium sized flask, put the remainder of the cells in 10-30mls of medium
4. Watch cells carefully over the first 24 hours and split as necessary
   If the cell line is adherant, after the viable cells stick, the medium can be removed and replaced with fresh medium