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 H_2O plus 0.35 g $NaHCO_3$
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
Alliquot 10 ml/tube and store at -20ºC

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 -20ºC

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 -20ºC for long term or 4ºC for short term

Sodium Bicarbonate = $NaHCO_3$
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 4ºC

FBS = Fetal Bovine Serum
Atlanta Biologicals cat # S11150
aliquoted 10 ml/tube and stored at -20ºC

XS = Xenopus Serum
Collected from frogs and stored at -20ºC
Spin in centrifuge before use to remove debri

TISSUE CULTURE MEDIUM

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

	1 Liter	2 Liters	3 Liters	4 Liters
IMDM	1 pkg	2 pkg	3 pkgs	4 pkgs
PS	10 ml	20 ml	30 ml	40 ml
NEAA	10 ml	20 ml	30 ml	40 ml
Insulin	10 ml	20 ml	30 ml	40 ml
2-Me	1 ml	2 ml	3 ml	4 ml
Primatone	3 ml	6 ml	9 ml	12 ml
$NaHCO_3$	3.02g	6.04g	9.06g	12.08

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

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 0.2um filter and store at 4ºC

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 4ºC

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 4ºC

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 4ºC

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 4ºC

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 4ºC

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 (Na_2HPO_4) 1.15 g/L	23 grams
Potassium Phosphate (KH_2PO_4) 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 4ºC

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 4ºC

RPMI 1640 Medium (For 1 Liter)

1 package of powder (Gibco cat #
10mls PS
1ml 2-Me
2g's $NaHCO_3$

pH to 7.0 with HCL
Filter thru 0.2um fllter and store at 4ºC

FREEZING and THAWING CELLS

PREPARE FREEZING MEDIUM:

50% Medium (appropriate for cell line being frozen)
50% FBS
To this add10% Hybridmax DMSO (Sigma cat # D-2650)
A batch can be made and aliquots can be frozen at -20ºC

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 N_2 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 N_2 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 10^6/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