REAGENTS FOR PREPARATION OF TISSUE CULTURE MEDIA

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FBS = Fetal Bovine Serum
Atlanta Biologicals cat # S11150
aliquoted 10 ml/tube and stored at -20^{o}C

XS = Xenopus Serum

Amph

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 4°C

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 4°C

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 -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 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