General Information for cell culture

A. Preparation

1) Media
   a. Add media (1 pack powder) in 900 ml of dd H_2O
   b. Dissolve $NaHCO_3$, antibiotic, and NAA with stirring
   c. Adjust volume to 1000 ml dd H_2O
   d. Adjust to pH 7.2 with 1 N HCl
   e. Sterilize by filtration through a 0.2 µm pore size filter under the clean bench
   f. Aliquot into 500 ml sterilized bottle and store 4°C
   g. Add serum and warm in 37°C water bath before use

2) Inactivation of serum
   a. Thaw serum at 37°C in water bath
   b. Heat at 60°C in water bath for 30 min
   c. Cool
   d. Aliquot into 100 ml sterilized bottle and store at -20°C
   □ serum may also contain substances inhibiting cell proliferation. Some of these maybe artifacts of preparation, e.g., bacterial toxins from contamination prior to filteration; the γ-globulin fraction may contain antibodies cross-reacting with the culture. Heat inactivation removes complement from the serum and reduces the cytotoxic action of immunoglobulin without damaging polypeptide growth factors.

3) Visblastine solution
   a. Dissolve vinblastine (10 mg/ml) in 100% DMSO and freeze at -20 °C for next use
   b. Add 100 µl vinblastine solution in 1000 ml media (1 mg vinblastine/1000 ml media)

4) Hormone-free serum
   a. Material : activated charcoal, Dextran 70, PBS
   b. Methods
      i. Prepare the charcoal-dextran solution (250 mg charcoal and 25 mg dextran/50 ml PBS)
      ii. Add 50 ml charcoal-dextran solution to 1000 ml serum (FBS)
      iii. Incubate at 55 °C in water bath for 30 min with stirring
      iv. Allow to cool
      v. Centrifuge or 10 min at 2000 rpm
      vi. Collect supernatant
      vii. Filter sterilize (0.2 µm) (1 filter for 100 ml serum)
      viii. Aliquot into 100 ml sterilized and store -20 °C

5) Hormone solution
   a. Estradiol for ZR-75-1
      i. Dissolve estradiol in ethanol and freeze at -20 °C for next use (314.4 µg estradiol/ml ethanol)
      ii. Add 10 µl of ethanol solution in 100 ml distilled water (3.144 µg estradiol/100 ml distilled water)
      iii. Filter (0.2 µm) sterilize and store at 4 °C
      iv. Add 0.5 ml of water solution in 500 ml of RPMI media (31.33 ng estradiol/1000 ml media, 0.1 nM)
   b. Testosterone for LNCaP
      i. Dissolve testosterone acetate in ethanol and freeze at -20 °C for next use (330.5 µg testosterone acetate/ml ethanol)
      ii. Add 10 µl of ethanol solution in 100 ml distilled water (3.305 µg testosterone acetate/100 ml distilled water)
      iii. Filter (0.2 µm) sterilize and store at 4 °C (33.05 ng testosterone acetate/1000 ml media, 0.1 nM)

6) 0.4% Sulforhodamine B (SRB)
   a. Dissolve SRB (0.265 g) in 500 ml acetic acid (1%)

b. Dye content approx, 75%

7) Phosphate-buffered saline (PBS)
    a. Dissolve NaCl (8 gm), KCl (0.2 gm), Na_2HPO_4 (1.15 gm) and KH_2PO_4 (0.2 gm) in 900 ml distilled water with stirring
    b. Adjust volume to 1000 ml with dd H_2O
    c. pH 7.4
    d. autoclave for 30 min on liquid setting
    e. cool liquid
    f. store at room temperature until needed

B. Procedure for freezing cells
1) collect cells
2) count cell number
3) concentrate the cell number ($1x10^6$ cells/ml media with 10% DMSO)
4) transfer 1 ml of cell solution into cryogenic vial
5) put vials into Nalgene cryo freezing container which containing isopropyl alcohol
6) keep the container at -70 °C overnight
7) transfer the vials into liquid nitrogen tank

C. Procedure for recovering frozen cells
1) add 15 ml of media into a 75 cm^2 tissue culture flask
2) thaw the frozen vial in water bath at 37 °C
3) spray 70% alcohol outside of vial for sterilization
4) transfer cells into tissue culture flask
5) change the media after 1 day incubation