LARGE SCALE MEDIA PREPARATION

1. Media Formulas for 20 liters.

A. Bicarbonate Buffered

Dulbecco's Modified Eagle's Medium (DME)
DME powder, 2 x 10 liter pkgs (267.4 g)
$NaHCO_3$, fresh bottle, 74 g.
H_2O, 18 l plus 1.9 l
CO_2 to pH 6.8

B. Hepes Buffered

i. DME-HEPES (10 mM)

DME powder, 2 x 10 liter pkgs (267.4 g).
NaCl, 50 g.
HEPES, 47.6 g.
H_2O, 18 l plus 1.9 l
Conc. HCl or 50% NaOH to pH 7.2

ii. Hank's-Hepes (10 mM)

Hank's powder, 2 x 10 liter pkgs (195.2 g)
HEPES, 47.6 g.
H_2O, 18 l plus 1.9 l
Conc. HCl or 50% NaOH to pH 7.2

iii. L15 + 10 mM HEPES + glucose (L15HG)

L15 powder, 2 x 10 liter pkgs (293.8 g)
HEPES, 47.6 g.
D-glucose, 40 g.
H_2O, 18 l plus 1.9 l
Conc. HCl or 50% NaOH to pH 7.2

2. Media Preparation: Add first portion of distilled or high quality H_2O to clean 5 gal container. Add chemicals with powder funnel. Use remainder of d. H_2O to rinse powdered media packets and funnel. Placing near pH meter, stir with large magnetic bar at least 2 hr for DME, 10 min for Hanks. Then adjust pH for A, bicarbonate buffer, by bubbling in 100% CO_2 through a dispo plastic 10 ml pipette, or B, HEPES buffer by addition of conc. HCl or 50% NaOH.

3. Filtration. Thoroughly tighten hose clamp and all connections on filter unit. Have sterile bottles ready to receive medium. Place medium on floor near laminar flow hood, attach pipette to inlet tygon tubing (into top of apparatus) and place in medium. Insert in pump. Place bell filter over a bottle. With bleed valve of filter open (up) pump until medium starts to run out and immediately close valve. Fill bottles, stopping pump between each one. Discard first bottle and number remainder in order of filling. Be sure to flame thoroughly neck and shoulder of each bottle before filling as the bell touches them. Use a two-hand motion of bell in left hand and moving bottles with right hand to avoid moving hand above bottles at any point. For bicarb buffer, all caps must have their sealer intact and rim of bottle must be undamaged. (For DME, after filling, place a 2 ml sample of each bottle in a 24 well plate. Place in incubator and test next day for growth and sterility by adding 0.4 ml of a cocktail prepared with 10 ml FCS, 1 ml 100 x gln, 1 ml Na pyruvate, 5 ml DME from previous batch, with no additives, vortexing, 3 ml of hybrid cell line, and vortexing.) Then cap tightly and wrap tape around bottom of cap. Store DME bottles in cold and dark (in boxes). (Fluorescent light causes toxic products to form). For Hank's, room temperature and light are permissible. Clean up same day--Rinse 5 gal carboy thoroughly 5 x with distilled water and dry before storage.

4. Millipore washing. Completely disassemble apparatus, rinse off all media with deionized water, and soak O/N in same. Next morning, rinse off and allow to dry. Follow millipore instructions for assembly and autoclaving.

Special notes: Wrap teflon tape twice around threads, clockwise with thread-end nearest you, before assembly.

Teflon O-rings have assumed shape of surfaces they are in contact with, i.e. are flat on filter side and bulged out at removal notch, so replace just as before. Tighten inlet, outlet, and bleed valve with wrench until moderate resistance is encountered. Tighten wing nuts by hand only moderately tight. Place hose clamp on inlet but only slightly tighten. (Further tightening is done just before use of filter unit).

Place paper bags over tubing and bell ends, coil up silicone tubing and wrap with 1/4 paper towel and then tape, and loop over wing nuts and tape in position.

Sterilize with bleed valve closed for directed length of time, 35 min. Longer will damage filter, so do not rely on automatic timer. Of course, remember to pre-run autoclave. After cooling, enclose filter unit in a plastic bag for storage.