Making Tissue Culture Medium

Procedure:

1. Autoclave either 12 one-liter or 20 half-liter bottles.
2. Let cool in the hood several hours
3. Wash and autoclave the media bucket and a large stir bar.
4. Autoclave 10 liter dH_2O
5. Place ~9 l autoclaved water in media bucket.
6. Stir in media powder slowly using a sterile stir bar. When all the powder is dissolved, rinse the bottle with sterile water and add to the solution. Keep the bucket covered with aluminum foil during stirring.
7. Add 3.7 g sodium bicarbonate per liter to media and dissolve by stirring. The media should be a deep red color.
8. pH the medium to 6.9 with 1 N HCL.
9. When finished pH'ing, bring the total volume to 10 L with sterile dH_2O.
10. In the tissue culture hood, set up the filtration pump. Sterilize the pump and tubing by running 1 liter of 10% bleach in dH_2O through it. Wash with several liters dH_2O to rinse away the bleach. Securely bolt an appropriate filter onto a ring stand using a hose clamp.
11. Filter the prepared medium into sterilized bottles at a pump setting of 4 or less. Fill each bottle to about 900 ml.
12. Take 1 ml aliquots from each bottle and transfer to 12-well plates. Grow for at least three days in the tissue culture incubator to check for growth.
13. Add penicillin/streptomycin from 1000X stock.
14. Close bottles tightly. Parafilm and label with media type, bottle number and date prepared.

Media Recipes for Different Cell Lines:

3T3	DMEM, 1% glutamine, 1% non-essential amino acids, 10% calf serum
A431	DMEM, 1% glutamine, 3% fetal calf serum, 7% newborn calf serum
KB	DMEM, 1% glutamine, 1% non-essential amino acids, 10% fetal calf serum
CHO	Ham's F12, 1% Glutamine, 10% fetal calf serum

Note: For DMEM, 20 mM HEPES is added to the medium while it is being made up.