

PREPARATION OF TISSUE CULTURE MEDIA

Media bottles are washed in automatic washer using first cycle with 1 second of soap and a second complete cycle without soap. Caps are rinsed thoroughly by hand in tap H₂O then distilled H₂O. Bottles are then autoclaved (30 min. on sterile cycle, 20 min. on dry cycle).

Wash down horizontal flow hood with 70% EtOH. Wash down outside surfaces of all material used in media preparation (ie. stirrer, pump, 20 L container).

Supplies needed:

- 20 L Nalgene vessel
- millipore peristaltic pump (model 7015-72)
- tubing to fit pump
- ring stand with clamp
- Millipak-40 filter unit (catalog # MPGL 04S H2)
- magnetic stirrer and large stir bar
- (Lamb lab has pump which we can borrow if making 20 L of media at a time)

Before starting, pump approx. 500 ml distilled H₂O through tubing. Add media powder slowly (to avoid clumping) to 9.5 L of distilled H₂O as per package directions. Then add NaHCO₃ as per package directions after powder is dissolved. When solution is complete, reduce stirring speed to low and filter 500 ml through Millipak filter unit into 500 ml flask. Note that you have to remove vent cover on filter unit when first starting to pump media through. Once media has displaced all air in filter unit, (ie. when media starts to flow through vent opening), start pump and replace vent cover.

When 500 ml has filtered through, adjust pH of the filtrate to 7.2 with a measured amount of 1N HCl. Add the 500 ml filtrate back to the vessel, add 19.5x the volume of 1N HCl used to adjust the pH, then adjust the total volume to 10 liter mark with H₂O. Then pump the final mix through the tubing for a few seconds before starting to fill bottles.

Flame the outside of each bottle neck before placing under the filter unit.

Vessel should be covered and media should be slowly stirring while media is being dispersed into bottles. If media is changing colors during dispersing, the pH may have to be readjusted during the time bottles are being filled.

When all bottles are filled, sample 2 ml out of 5 randomly selected bottles and allow these samples to incubate for 5 days in CO₂ incubator to check sterility. Cap bottles very tightly and store at 4°C.

Vessel should be rinsed thoroughly with tap then distilled H₂O. Pump distilled H₂O through tubing and filter unit. Filter unit can be wrapped in foil and autoclaved (remove plastic). Filter unit will be good for 2 additional uses.