

Generation of B95-8 EB-virus

Protocol

- Grow B95-8 cells up to 1L in RPMI (+10%FBS, +Pen/Strep) at 37°C, 5% CO₂ (in aerated T150 flasks).
- When media starts to turn yellow, let cells grow another 10-14 days.
- Spin out cells in sterile centrifuge tubes (glass bottles for RC3B are fine)
- Pass through a 500ml 0.8 micron filter (Fisher, cat #0974025AA) [note: put an autoclaved pre-filter (Fisher Cat #0987446C) on top of the 0.8 micron filter to catch the remaining cells plus the bulk of debris (this helps prevent the 0.8 micron filter from clogging)].
- Transfer to sterilized 250ml centrifuge tubes and spin at 12k for 2.5 hrs [note: the rotor is used to spin bacteria and there is usually a lot of junk in it, so rinse with 70% ethanol prior to use. In addition, after spinning, put tubes on a diaper in tissue culture hood (this should help prevent contamination of hood) and squirt tops of tubes with 70% ethanol prior to opening].
- Decant media and suspend in a total of 10ml of media (use media that is used culture the recipient cells (i.e. the cells that will be infected) and transfer to 50ml tube.
- Add another 5ml of media to first centrifuge tube, pipette up and down a few times, transfer to next centrifuge tube, pipette up and down a few times, etc. After washing all tubes, combine with the first 10ml of virus.
- Transfer 1ml aliquots to sterile cell freezing tubes and store at -70°C.