Growth of B95.8 Cell Line and Production of Human EBV Virus

Note: this protocol requires the use of a sterile tissue culture environment, as well as sterile materials and media. This process takes over 2 months, so EBV media use should be carefully monitored. Additionally, proper PPE should be worn at all times, removing them before leaving the lab area.

Decontaminate of any pipets used to with the virus containing media by placing them in the pipet chimney that contains 10% Clorox solution. Once the pipets have been decontaminated, they can be removed from the chimney, drained of liquid and disposed of in a biohazard box.

Once you are done, decontaminate the collected liquid waste by adding 10% Clorox solution and allow to sit for 15 minutes. After 15 minutes, the decontaminated waste may be flushed down the sink with water.

Day 1

1) Pre-warm complete RPMI-1640 media to 37°C.
2) Partially thaw cryo-preserved B95.8 cells by gently agitating cryovial in 37°C water bath. Proceed to the next step before cells are completely thawed. A face shield should be worn for the thawing process.
3) In a sterile tissue culture hood, spritz the vial of cells with 70% EtOH.
4) Using a 5 ml pipet, *slowly* add 1 ml of FBS to half-frozen cells.
5) Add the 2ml FBS/cell mixture from step 4 to a T75 flask containing 18 ml of complete RPMI.
    a. Monitor cells for growth. Note there ARE some differences from lymphoblasts:
    b. B95.8 cells do not grow in clusters.
    c. B95.8 cells grow more aggressively than lymphoblasts. They tend to double about every 24 hours, once in log-phase.
    d. B95.8 cells are intolerant of acidic conditions – meaning you must keep close watch to ensure that they don't get too acidic.

Day 2

6) Add 20ml of fresh media. You will now have 40ml total of culture

Day 3

7) Add 20ml of fresh media. You will now have 60ml total of culture.

Day 4

8) On the 4th day, split the culture into 2 x T75 flasks, adding 30ml of culture to each. Add an additional 20ml of fresh media. You will now have 50ml total of culture in each flask.

Day 5

9) Add 20ml of fresh media to each flask. You will now have 70ml of culture in each flask.

Day 6

10) On the 6th day, split each T75 flask into 7 x T150 flasks, adding 10ml of culture to ach flask. Add 20ml of fresh media to each flask. You will now have 30ml of culture in each flask.

Day 7

11) Add 30ml of fresh media to flask. You will now have 60ml of culture in each flask.

Day 8

12) Add 30ml of fresh media to each flask. You will now have 90ml of culture in each flask.

Day 9

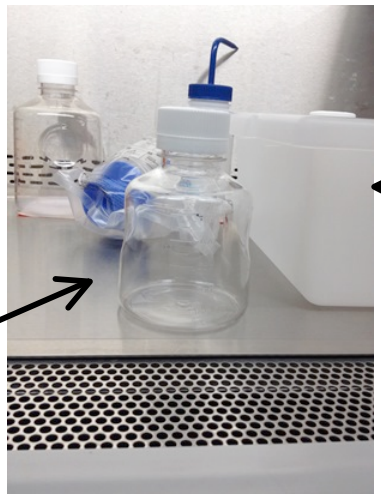
13) Add 40ml of fresh media to each flask. You will now have 130ml of culture in each flask. Carefully lay each flask flat.

Day 10 - 20

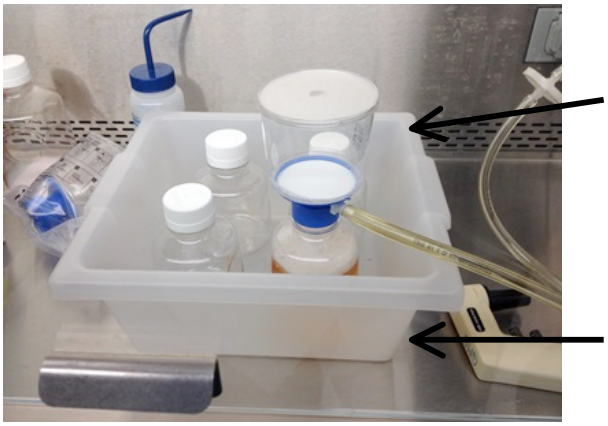
14) Do nothing.

Day 21 – Harvest Virus Conditioned Media

15) Carefully aliquot the culture into 50ml conical tubes, putting 45ml of culture into each. Decontaminate flasks by the addition of 10% Clorox solution.
16) Remove the cells by centrifugation (1,400 x g for 15min).
17) Carefully pool the cleared culture into 4 x 500ml Filter Receiver and Storage Bottles (Thermo, cat #455-0500). Make sure the bottles are in a secondary container to contain any spills. Decontaminate cell pellets by the addition of 10% Clorox solution.



18) Next filter the culture through a 0.45um filter, fitted into a new 500ml Receiver and Storage Bottle (Filter – Thermo, cat #295-4545). Do this for each pooled batch, being sure to keep them in the secondary container. Decontaminate all used filters and receptacles by the addition of 10% Clorox solution.



19) Filter a second time through a 0.22um filter into a new 500ml Receiver and Storage Bottle (Filter – Thermo, cat #595-4520). Again, receivers should remain in secondary container. Decontaminate all used filters and receptacles by the addition of 10% Clorox solution.
20) Once filtered, aliquot the culture into workable volumes (10ml -20ml) in conical tubes. Label each with "EBV" and the date. Store at –80°C, in a secondary container, in a locked freezer.
21) Pull out batches as needed for initiation and store at 4°C in a secondary container. Mark off the aliquots on the inventory sheet as they are used.

**Do not use EBV media that has been thawed for more than 48 hrs.

*See document **Virus Growth Schedule** for a day-to-day schedule for virus production.*

RPMI-1640 (complete)

500 ml of RPMI-1640
100 ml heat-inactivated FBS (20%)
6 ml antibiotic-antimycotic (1x) – Gibco/Invitrogen: Cat. No. 15240-062
1 ml Tylosin solution – Sigma-Aldrich: Cat. No. T3397