B cell Transformation and Care

Item	Manufacturer	Order Number
RPMI-1640	Sigma	R0883
Pen/Strep (5000 IU Pen/ 5000ug/mL Strep)	Cellgro	30-001-Cl
L-glutamine (200mM; 29.2 mg/mL)	Cellgro	25-002-Cl
HEPES (1M; 238.3mg/mL)	Cellgro	25-060-Cl
FBS, Heat-inactivated	Sigma	F4135 (we have tested lot for the lab)
PBS	Sigma	D8537
15 ml propylene tubes	Falcon	352063
24 well plate	Corning	3524
T25 flasks with 0.2 ul filter	Corning	3056
T75 flask with 0.2ul filter	Corning	430641
CSA	Sigma	

1. Separate fresh blood with Ficoll Hypaque. See SOP#18 for details. Or use a frozen 10M PBMC aliquot. See SOP#8 for Thawing.
2. Count using a 10-fold dilution.
3. Measure 10M PBMC.
4. Spin cells in 15 ml conical , aspirate to about 200ul and add:
   a. 1.5 ml FCS
   b. 1.5 ml EBV stock (you can find it -80)
   c. 1 ml R10
   d. Add 4ul of our 1mg/ml stock of CSA (-20 B3 lab). Final concentration should be 1μg/ml. CSA (cyclosporine) inhibits T cells.
   e. Mix the mixture and split to 2 wells on 24 well plate – 1 well will have 5M cells.
   f. Add sterile PBS in surrounding wells
   g. Do not put more than 2 different subjects on 24 well plate (must be separate by PBS to avoid cross contamination)
   h. Discard left over CSA and EBV Sup. Do not refreeze!
5. Feeding:
   a. 10 days after transformation aspirate off most of the media in the wells without removing cells. Add back an equal amount of R20 (20% FBS in RPMI + P/L + Hepes) and mix gently.
   b. Continue to feed wells in this way twice a week. (Aspirate w/o removing cells, and add back R20).
   c. When the wells are very dense (look under microscope), and only if there is only one patient on the 24 well plate, split the two wells to four wells and add R20 to bring the volume up. (If there are two patients on the plate, do not split).
   d. After 6-8 weeks (about how long cell transformation takes) 2 wells might be transferred to T25 flask and the remaining 2 split to two wells. Add 4ml R20 to T25 and mix. Maintain the plated cells for a few weeks until you are certain your T25 flask is growing well.
   e. Feed T25 flask R20 twice a week by medium exchange until cells appear dense on the bottom of the flask. Always examine the flask to be fed (split) for macroscopic appearance (fungus/bacteria contamination). Media should appear yellow (exception: K562 cells tend to grow more basic and may appear orange or even pink). Cells should be growing in clumps

f. Mix the contents of the T25 flask gently. Leave 1ml in the T25 and transfer 5ml to a T75 flask. Add 4ml R20 to the T25 (1:5 feeding) and add 10ml R20 to the T75. Continue feeding the T25 1:5 with R20 twice a week (this is your backup supply).
g. Feed the T75 1:4 twice a week with R20 by media exchange. Try to keep the concentration at 1M/ml, and no more than 20M cells in one T75. Count the cells periodically and split to more T75s when necessary. To split a T75, mix and move half to a new flask. Feed each new flask 1:1 with R20. T75s are the flasks you will freeze from.
h. Feed T75 1:1 with R20 the day before you plan to freeze to boost viability.
i. Try to freeze 10 vials of 10M/ml for each subject. Viability MUST be **>80%**. See protocol for freezing SOP# 07.

Improving Viability:

- If cell concentration is high, but viability low, split to two flasks or discard some of the cells to encourage proliferation of new ones.
- If concentration is low and viability is low, or if your cells just do not seem to be proliferating, spin down the cells and resuspend them at 1M/ml in fresh R20.

5. If the Bcells are cultivated and kept in the incubator for more than 2 months – do macoplasma testing. See SOP#13.
6. Infection: Prior to vaccinia infection, do not discard any cells. Instead add media in the ratio of 1:1 on the day before infection. You can discard some cells if you don't need a lot of vaccinia targets--plan before your assay how many targets are needed.