

## CTL feeding

### Reagents

Reagent	Vendor	Catalogue #
RPMI-1640 media	Sigma	R0883
L-Glutamine	Mediatech	25-002-C1
Pen/Strep (5000IU Pen/5000ug/ml strep)	Mediatech	30-001-C1
FBS	Sigma	Actual tested in PARC lo
IL-2	NIH AIDS Research &Reference Reagent Program	Cat#136

1. CTL's are fed twice a week, usually on Mon/Fri by medium exchange. The medium is R10/50 with PLGH (penicillin, L-glutamine, and Hepes buffer) and IL-2 at 50U/ml .
2. Remove flasks from the incubator very carefully to avoid disturbing the CTLs growing on the bottom and place them in the hood. Feed 5-8 flasks at a time to reduce the time CTLs have to sit at room temperature. Usually there is about 20 ml in the T-25 flask. Aspirate 10 ml off the top with the vacuum aspirator slowly and gently to avoid taking any cells from the bottom of the flask. Add 7 ml of fresh R10/50 medium. Make sure the medium is not discolored—if it is, the pH balance is probably not correct and your cells might not like it at all! If it appears discolored, then don't use it. R10/50 IL-2 media CAN NOT be older than 2 weeks !
3. Change gloves every time you exit the hood and always put on a fresh pair before you enter the hood again!
4. CTLs must be checked for activity after each restimulation with current standard assay (Elispot , Cytotoxicity assay , flow cytometry )
5. If you have a big and constant demand on a particular clone it is a good idea to restimulate it every week. After the 3<sup>rd</sup> week, take an aliquot from the old flask, put it into a new flask and restimulate. After the 4<sup>th</sup> week, restimulate the one you did two weeks ago etc.
6. For freezing, use cells that have been in culture for about 2-3 weeks and are in good shape and have good viability. We usually freeze them at 4-5 million per vial.
7. Active clones stay active as long as they are in the flask .They don't proliferate after 3 weeks but if fed are good for experiments as long as they are there.
8. If you are done with a clone, then freeze it down! Do not waste any clones!
9. It is also important to be careful with the incubator during feeding! Prolonged amounts of time with open doors can change the temperature and CO<sub>2</sub> levels, which is very disturbing to CTLs!
10. If you have 20 CTL cultures, do not take them out of the hood all at once. Try to minimize their time outside their preferred conditions by feeding them in smaller groups of 5-8.
11. Try to keep your CTLs in an incubator separate from cultures and experiments that need to be removed often. Keeping your CTLs in a separate incubator from the transients will also minimize their temperature and CO<sub>2</sub> discomfort.

**Check incubator CO<sub>2</sub> and temperature levels once a week with the FYRITE kit.**