| STANDARD OPERATING PROCEDURE | | | | |
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| Ragon Institute of MGH, MIT and Harvard | CTL Feeding | | **SOP#** | TC-SOP-004 |
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## I. PURPOSE

The purpose of this procedure is to outline the CTL Feeding assay performed in Ragon laboratories.

## II. SAFETY

This procedure needs to be carried out in the BSL2+ laboratory following all BL2+ regulations.

## III. SCOPE

This procedure applies to all employees, students, contractors and visitors that work on the CTL Feeding protocol.

## IV. REQUIREMENTS

Training to be obtained by qualified trainer or Subject Matter Expertise (SME).

## V. RESPONSIBILITIES

A. The Ragon Institute **qualified trainers** are responsible for the overall implementation of this procedure and ensuring compliance and for periodic review of this procedure. Updates if any may be initiated by the qualified trainers or Subject Matter Expertise (SME).
B. **All employees, students, contractors and visitors** are required to follow this procedure.

## VI. 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 |
| IL-2 | NIH AIDS Research &Reference Reagent Program | Cat#136 |

## VII. PROCEDURE

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 must 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 clone it is a good idea to restimulate it every week. After the 3$^{rd}$ week, take an aliquot from the old flask, put it into a new flask and restimulate. After the 4$^{th}$ 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 if they are in the flask. They don't proliferate after 3 weeks but if fed they stay good for experiments.

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 $CO_2$ levels, which are very disturbing to CTLs!. Make sure the thermometer is in the incubator.

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 $CO_2$ discomfort.

12. Clones like to grow at 1-1.5million/ml. Feed accordingly and split if necessary.

**Check incubator $CO_2$ and temperature levels once a week with the FYRITE kit.**