



**Title:** CTL Secondary Restimulation Assay for BALB/c  
Mice ( $H-2^d$ )

No: RTLP-GL-FA-14

Location:  
Old CCRC Tripp Lab

Approval Date:  
22 November 2005

Supersedes Date:

**Materials:**

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- |                                |  |   |                  |
|--------------------------------|--|---|------------------|
| •Lab coat                      | •HBSS                                  | •Gamma Cell (for<br>Irradiation)                          | •Pipettes        |
| •Gloves                        | •Turk's stain                          | •dH <sub>2</sub> O  | •Pipetteman      |
| •Prepared P815 target<br>cells | •37°C, 5% CO <sub>2</sub><br>incubator | •DMEM, 5% FBS<br>supplemented with Tumor<br>Cocktail (TC) | •Pipette Aid     |
| • Prepared spleen              | •Centrifuge                            |   | •Pipetteman tips |
|                                |  |   | •24-well plates  |
|                                |  |   | •hemacytometer   |

**Procedure:**

- 1) **Prior to assay begin growing P815 cells**
- 2) **24h prior to assay, prepare 'stimulator' P815 cells:**
  - a) Infect P815 cells with RSV to achieve a MOI ~ 10
  - b) Use serum-free media in all phases of infection
  - c) Goal is to achieve  $2 \times 10^7$ /ml RSV-infected P815 cells – calculate total volume needed based on 24 well plate format used, e.g. if you use 12 wells, then you'll need 2.4 ml at  $2 \times 10^7$ /ml or a total of  $4.8 \times 10^7$  cells
  - d) Infect overnight at 37 °C
- 3) Aseptically remove a spleen and prepare to a single-cell suspension
- 4) Wash 1X with HBSS or media
- 5) Count for viable cell numbers using Turk's (eliminates RBCs)
- 6) Resuspend cells in "TC media" to  $2 \times 10^6$  cells/ml – leave on ice
- 7) Remove RSV-infected P815 cells from incubator
- 8) Wash, resuspend in "TC media", gamma-irradiate at 2500 RADS
- 9) Wash and resuspend in "TC media" to final volume needed for assay

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- 10) Using a 24-well plate, add 2 ml of diluted spleen cells/well ( $4 \times 10^6$  cells/well)
- 11) Add 0.2 ml of RSV-infected, gamma-irradiated stimulator cells
- 12) Culture for minimum of 5 days at 37<sup>o</sup> C – usual harvest is at day 6 post restimulation

CTL Assay:

- **One day prior to harvest of secondary re-stimulated lymphocytes**, prepare P815 target cells that are RSV-infected as noted above
- Use non-radioactive CTL assay protocol for labeling target cells
- On day of harvest, pool all wells of 24 –well plate containing re-stimulated CTL
- Wash cells in DMEM-5% FBS
- Resuspend accordingly for non-radioactive CTL assay and begin with that protocol