



Title: ADCC and NK Cell Assays of BAL Cells

No: RTLP-FA-1

Location:
Old CCRC Tripp Lab

Approval Date:
10 September 2004

Supersedes Date:

Materials:

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| •Lab coat | •Dulbecco's | •Target Cells (in log | •Antibodies: |
| •Gloves | Phosphate-Buffered | phase): YAK, P815, | B220, HB197 |
| •Centrifuge | Saline (D-PBS) | P815-GA, P815- | •Mini-tubes |
| •Incubator 37°C | ⁵¹ Cr | GB, P815-F | •Pipettes |
| •Minimal Essential | •96-well U-bottom | •Dynal Magnetic | •Pipetteman |
| Medium (SMEM) | microfuge plates | beads (anti- mouse | •Pipette Aid |
| •BSA | •15mL tubes | IgG) | •Pipetteman tips |
| •Ice bucket w/ice | •50mL tubes | •Dynal Magnet | •4°C Cold Storage |

Procedure:

I. Preparation of Target Cells: (18h before assay)

1. Expand YAK cells (NK cell targets), or P815, or P815 subcloned cell lines (P815-GA, P815-GB, P815-F)(ADCC targets) in log phase to ensure you have enough target cells for assays.
2. Into a 15mL tube, aliquot 10^6 target cells in 1 ml of SMEM.
3. Pulse with 200 μ Ci ⁵¹Cr and incubate at 37°C for 6h or overnight (18h).
4. Following the incubation, wash cells 1X with SMEM and resuspend in 5 ml of SMEM+10% FBS (TCM).
5. Count cells and dilute target cells to 2×10^5 cells/ml in TCM.

II. Preparation of Effector Cells: From mouse bronchoalveolar lavage (BAL)

Wash the lungs of 5 mice with 1% BSA/PBS and pool contents (see RTLP-GL-CP1)

1. Centrifuge cells harvested from BAL in a 50 mL tube and discard supernatant.
2. Resuspend cell pellet in 1:200 anti-B220 Ab diluted in D-PBS
3. Incubate on ice for 30 minutes, resuspend by flicking bottom of the tube every 10 minutes to ensure cells remain suspended in antibody solution.
4. While effector cells are incubating, prepare anti-mouse Ig Dynabeads by re-suspending 200 μ l of beads in D-PBS in a 15 ml tube, and placing against the magnet.

5. Pour off the D-PBS wash.
6. Resuspend beads in 5 ml of D-PBS.
7. Centrifuge anti-B220-coated BAL cells
8. Resuspend in washed Dynal beads, and incubate for 30 minutes at 4°C; Be sure to rock samples every 15 minutes to ensure maximal bead/cell contact.
9. Place 15 mL tube against magnet to remove beads and B220⁺ cells.
10. Collect non-magnetic cell eluate into a 15mL tube.
11. Centrifuge eluate and resuspend in 2 ml of TCM.
12. Count cells and dilute cells to 6×10^5 /ml.

III. YAK Target Cell Assay

1. Remove 1 ml of effector cells and make 1:3, 1:6, 1:12 dilutions in TCM
2. Add 100 µl/well in triplicate – be sure to leave a set of triplicate wells for spontaneous release and maximal release on the 96-well U-bottom plate
3. Add 100 µl /well of Cr⁵¹-labeled YAK targets to all wells
4. Add 100 µl /well SMEM for spontaneous release; however, add nothing to maximum release wells.
5. Gently centrifuge plate for 3 minutes at 1200 rpm then incubate for 5h at 37°C.
6. Harvest supernatant into mini-tubes, i.e. 1.5 ml polystyrene tubes in a 96-well format (BioRad) for radioactive analysis.

IV. P815 Target Cell Assay

1. Centrifuge 1 ml of effector cells and resuspend in 1:10 dilution of 2C11 (anti-CD3 antibody) in D-PBS.
2. Incubate for 30 minutes on ice.
3. Centrifuge cells at 500xG for 15 min at 4°C, discard supernatant, and resuspend to 6×10^5 cells/ml in TCM
4. Make 1:3, 1:6 and 1:12 dilutions in TCM.
5. Add 100 µl /well, taking care to set up plate as shown above for YAK cells.
6. Add 100 µl /well of radiolabeled P815 target cells, 10^5 cells/well.
7. Gently centrifuge plate for 3 minutes at 1200 rpm then incubate for 5 hours at 37°C.
8. Harvest supernatant into mini-tubes for radioactive analysis (gamma counter).

| Author | Management Approval/Date | Effective Date |
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