SOP 06-04 Macaque NK cell assay (nonradioactive) by calcein release

Purpose and principle:

This assay measures macaque NK cell-mediated lysis of K562 cells using the release of calcein, measure by fluorometry, as the assay endpoint.

Materials:

- K562 cell line
- Calcein AM *special packaging* (Molecular Probes #C-3100MP)
- Pluronic F-127 *20% solution in DMSO, (Molecular Probes #P-3000)
- RPMI 1640 medium without phenol red
- Fetal bovine serum (FBS)
- 2% Triton X
- Heparinized blood from *M. mulatta*
- Ficoll-hypaque (Amersham-Pharmacia #17-1440-03)
- Phosphate buffered saline, pH 7.2
- 0.3 ml 8 tube strips with domed caps, (Continental Lab Products #3441)
- 14 ml polypropylene tubes, (Corning)
- 96 well Wallac plate
- 96 well Black Optiplate^{TM} 96F (Perkin Elmer #6005270)

Procedure:

A. Preparation of target cells
   1. Make a 1 mM solution of calcein AM by reconstituting 50μg of calcein AM in 25 μl DMSO/25 μl Pluronic F-127
   2. Label 10^6 K562 cells in 1 ml RPMI 1640/10% FBS with 10 μl 1 mM calcein AM
   3. Incubate for 90 minutes at 37^{o}C
   4. Wash 2X with 13 ml RPMI 1640
   5. Resuspend at 10^5 cells/ml in RPMI 1640/10% FBS

B. Preparation of effector cells
   1. Layer 5 ml of blood over 5 ml ficoll
   2. Centrifuge at 1000g for 20 minutes
   3. Aspirate mononuclear cells at ficoll/plasma interface
   4. Pellet cells at 500g x 7 minutes
   5. Resuspend cells in 5 ml NH_2Cl_4 for 5 minutes to lyse RBCs
   6. Add 7 ml PBS and pellet cells at 500g x 6 minutes
   7. Resuspend in RPMI 1640/10% FBS
   8. Adjust cell concentration to 10^7/ml

C. Cell mixing

1. In 0.3 ml 8 tube strips, add:

Effector:Target Ratio	Effector cells	Target cells	Medium
100:1	100 μl	100 μl	0 μl
50:1	50 μl	100 μl	50 μl R10
25:1	25 μl	100 μl	75 μl R10
12.5:1	12.5 μl	100 μl	87.5 μl R10
control (spontaneous)	0 μl	100 μl	100 μl R10
control (maximal)	0 μl	100 μl	100 μl TritonX

2. Spin strips in a 96-well Wallac plate at 800 RPM for 3 minutes
3. Incubate plate at 37^{o}C for 4 hours
4. At the end of the incubation, spin the plate at 1400 RPM for 8 minutes and harvest 100μl of supernatant from each tube into a 96 well Black Optiplate^{TM} 96F.
5. Read the plate on a fluorometer, at excitation wavelength 494 nm, emission wavelength 517 nm.

D. Calculations

Percent specific lysis for each E:T ratio is calculated using the following formula:

$$\frac{\text{Experimental release} - \text{Spontaneous release}}{\text{Maximal release} - \text{Spontaneous release}} \times 100$$

E. References

Choi EI, Peterson L, Reimann KA. A fluorometric assay for measuring NK cell activity in macaque monkeys. Submitted, 2006.

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