

## SOP 07-02 Macaque T cell activation using anti-CD3

### Purpose and principle:

Method to activation macaque T cell in vitro by cross-linking T cell receptor using plate-bound anti-CD3 monoclonal antibody

### Materials:

- 1 x sterile PBS
- Anti-macaque CD3 mAb: 6G12 or FN18\*
- Complete RPMI-1640 or other cell culture medium
- Isolated macaque PBMC
- 6 well plate

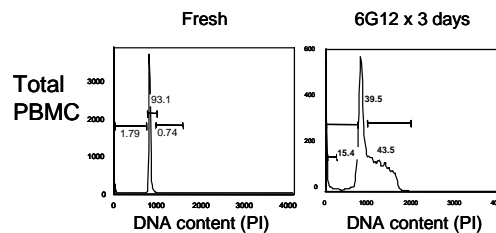
\*both antibodies available through the *NIH Nonhuman Primate Reagent Resource*

### Procedure:

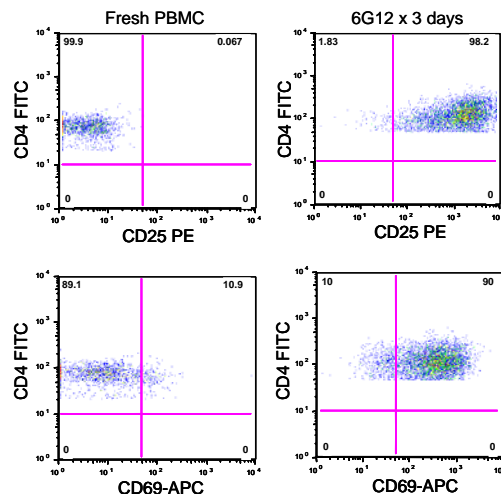
1. Prepare a 10ug/ml solution of anti-CD3 in sterile PBS
2. Dispense 2ml of antibody solution to each well of the 6 well plate,
3. Incubate plate at 37°C for 2 hours or plate one day in advance at 4°C
4. Aspirate antibody solution.
5. Rinse each well with PBS twice
6. Resuspend macaque PBMC in RPMI-1640 or other complete medium at  $1-2 \times 10^6$ /ml.
7. Add 3-5 ml ( $3-6 \times 10^6$  cells) to each well and incubate plate at 37°C, 5% CO<sub>2</sub> incubator for 2-4 days.

### Example of rhesus T cell activation using 6G12:

Activation shown by cell proliferation:



Activation shown by induction of CD25/CD69 expression:



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Date: 26 March 2007  
Revised: