# SpheroTECHNICAL NOTES

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## Separation of Mononuclear Cells from Peripheral Blood using SPHERO™ Goat anti-mouse IgG Magnetic Particles

Materials:

1. Gt anti-Ms IgG magnetic particles, 1% w/v, Cat. # MM-40-10 or MMXA-40-10, ~$2x10^8$ particles per ml.
2. Appropriate monoclonal antibody (anti-CD3, CD4 or CD8 etc.)
3. Appropriate FITC conjugates
4. Dulbecco PBS
5. Fetal bovine serum
6. Histopaque
7. Paraformaldehyde fixative
8. FlexiMag Separator, Jr.

PROCEDURES:

SEPARATION OF MONONUCLEAR CELLS FROM PERIPHERAL BLOOD

Peripheral blood was collected by venipuncture of antecubital vein. The blood was drawn into heparin Vacutainer tubes, transferred to 50 ml polypropylene centrifuge tubes and diluted with an equal volume of calcium and magnesium-free Dulbecco's phosphate buffered saline.

Ten to 20 ml aliquotes of diluted blood were layered onto an equal volume of Histopaque in 50 ml tubes, and centrifuged for 30 minutes at ambient temperature at a centrifugation force of 400g at the blood/Histopaque interface. The lymphocyte band was aspirated into a 15 ml centrifuge tube and the volume brought to 14 ml with Dulbecco PBS containing 2% v/v heat-inactivated fetal bovine serum, or 5% plasma protein fraction. The cells were pelleted by centrifugation at 4°C for 7 minutes at 450g, washed with 14 ml of

PBS, and recentrifuged. The final cell pellet was resuspended in 1-2 ml buffer, and a viable cell count performed. Only cell preparations with a viability of >95% were used.

## COATING OF ANTI-MOUSE IgG MAGNETIC PARTICLES WITH MONOCLONAL ANTIBODY

Add 2 to 5 μg of monoclonal antibody to 100 μl of 1% w/v Gt anti-Mouse IgG coated magnetic particles (~$2x10^7$ particles). in 12x75 tubes. Incubate the mixture with occasional mixing, at 4 °C for at least 30 minutes. Wash the particles three times with 2 ml of Dulbecco PBS.

## INCUBATION OF MAGNETIC PARTICLES AND CELLS

Pipet ~$2x10^6$ cells into 12x75 mm glass test tubes and add monoclonal antibody coated magnetic particles to the tubes at the particle to cell ratio of 5~20 to 1. Incubate the tubes for at least 30 minutes at 4 °C on the rotator at a speed setting of 2 to 4 rpm. Separate the magnetic particles with FlexiMag Separator or FlexiMag Separator,Jr. Transfer the supernatant liquid, containg the unbound cells to a 12x75 mm tubes and count the cells in a hemacytometer, and/or analyze by immunofluorescence.

## DETECTION OF CELL DEPLETION BY IMMUNOFLUORESCENCE

Determine the proportion of target cells in the mononuclear cell suspension pre- and post-separation by immunofluorescence staining and examine visually or by flow cytometry.

Incubate the remaining cells (unbound cells) after immunomagnetic separation with 40 μl of anti-target cell monoclonal antibody for at least 30 minutes at 4°C with occasional mixing. Wash the cells three times with 2 ml of the buffer. Resuspend the cell pellet with 50 μl of a 1/10 dilution of FITC-conjugated anti-mouse IgG antibody in PBS/FBS buffer. Incubate and wash the cells as described previously. Resuspend the final cell pellet in 300 μl of paraformaldehyde fixative and store at 4°C in the dark, until being analyzed on the flow cytometer or fluorescence microsope.

Perform the visual detection using a fluorescence microscope. Examine the cells under normal illumination and count the number of cells. Re-examine the same field under epi-illumination, and count the number of fluorescent cells. Categorize at least 200 cells in each preparation.

For flow cytometer evaluation, calibrate the flow cytometer and establish the lymphocyte gate based on the forward/side scatter profiles, or by back-gating according to the manufacturer's directions. Classify at least ten thousand cells from each tubes and analyze the data using appropriate software.

Controls should consisted of mononuclear cells incubated with (i) monoclonal antibodies to obtain pre-depletion target cell percentages; (ii) particles coated with the isotype control monoclonal- to determine non-specific depletion of the target cells by the particles; (iii) each of the post-depletion analysis reagents independently to evaluate background binding of these reagents; (iv) the Leucogate reagent for cytometric analysis to determine the purity of the mononuclear cell preparation and the accuracy of the gating procedure.

Calculate the percent depletion as:

$$\frac{\%\ \text{Target cells post-depletion}}{\%\ \text{Target cells pre-depletion}} \times 100$$

NOTE: 1. For cell separation using 1~2 um magnetic particles at 0.25% w/v, such as MMXA-10-10 or MMX-10-10, use the same vol as in MMXA-40-10 or MM-40-10 at 1% w/v.