With over 35 million people infected with the Human Immunodeficiency Virus (HIV) and infection rates continuing to rise, the need for better understanding of the viral infection, monitoring and treatment is high. Magnetic separation technology, such as Dynabeads®, is an ideal tool for studying HIV infection and related AIDS research.

Dynabeads® have been used in many ways to increase the understanding of the virus targets and the effects on the immune system.

It is becoming increasingly clear that HIV infection affects not only CD4+ T cells, but also phagocytic cells expressing the CD4 antigen including macrophages, follicular dendritic cells and Langerhans cells. Furthermore, the virus is able to enter cells expressing receptors such as CCR5 and CXCR4, which includes a subset of natural killer (NK) cells (1). This suggests that for further HIV and AIDS research many different populations of cells need to be studied.

**Cell Separation**
Dynabeads® provide a simple and rapid system for the separation of any cell population from whole blood or prepared samples such as mononuclear cells (MNC). Due to the properties of the magnetic beads and the efficient tube-based system, high cell viability, purity and yield is obtained (see fig. 1). Isolated cells can be used in the downstream application of your choice and examples are outlined in the table overleaf.

**Cell Expansion**
Due to the size of Dynabeads® (4.5µm) being similar to white blood cells, the beads can be coupled with cell signalling molecules and used to mimic in vivo cell interactions (see figure 2) (2). Over 1000 fold expansion of T cells can be achieved in 12 days when naïve T cells are incubated with Dynabeads® CD3/CD28 T Cell Expander (see fig. 3).

**Cell Counting**
Dynabeads® can be used to determine the number of CD4+ and/or CD8+ T cells in whole blood samples using a simple 30 minute protocol. Step one involves depletion of monocytes using Dynabeads® coated with anti-CD14. To capture the CD4+ or CD8+ cells, Dynabeads® coated with anti-CD4 or anti-CD8 are added. The final step involves lysis of the captured cells and counting of the nuclei. The method is accurate and reliable and correlates well with flow cytometry (3).

Fig. 1: Cell separation using Dynabeads®. Dynabeads® are added to a starting sample (blood, bone marrow, buffy coat, MNC, cell suspension) in a tube. The beads bind to the target cells in a 15-30 minute incubation and the tube containing sample and beads is placed in a magnet (Dynal MPC®). The beads and bound cells migrate to the magnet and the supernatant is removed with a pipette. Dynabeads® can be detached from isolated cells if required. Cells isolated with Dynabeads® can be used in many assays/applications including cell culture, functional and proliferation studies, flow cytometry, molecular studies, cytokine secretion, phenotyping and further sorting.

Fig. 2: Dynabeads® signalling to T cells

Fig. 3: Expansion of T cells from peripheral blood
CD4+ T cells
Positive isolation and detachment of CD4+ T cells from MNC using Dynal®CD4 Positive Isolation Kit.

CD4+, CD28+
Long term survival of HIV infected CD4+ T cells

CD4+, CD7-
Characterisation of a T cell subset and the role in HIV infection

CD4+, CD56+
Assessment of intracellular glutathione redox status

CD8+ T cells
Studies of HIV-suppressive factors

CD8+, CD28-
Studies on the costimulatory effect of the CD28 antigen on HIV replication

CD8+ cells
Isolation of subpopulations: 96% pure CD8+, CD8RA- or CD8-, CD4RA+ cells were obtained. CD8+ and CD4+ populations were obtained from MNC using Dynal® Positive Isolation Kits. The bead-free cells were further sorted into RA- or RO+ with respective antibodies coated onto secondary Dynabeads®. CD8+, CD8RA- cells were also negatively isolated from MNC by depleting CD8, CD14, CD19, CD6 and CD8RA with antibody coated Dynabeads®. The negatively isolated cells showed equivalent results to positively isolated cells.

NK cells
Development of a whole blood NK cell assay to assess NK activity in viral disease

References
7. (Aust)
8. (NZ)

Ordering Information

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Product Use</th>
<th>Product Number</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynal® CD4 Positive Isolation Kit</td>
<td>To positively isolate and detach cells. Contains Dynabeads® and DETACHaBEAD®</td>
<td>113.03/113.04</td>
<td>2ml/10ml kits</td>
</tr>
<tr>
<td>Dynal® CD8 Positive Isolation Kit</td>
<td>To positively isolate and detach cells. Contains Dynabeads® and DETACHaBEAD®</td>
<td>113.05/113.06</td>
<td>2ml/10ml kits</td>
</tr>
<tr>
<td>Dynabeads® CD4</td>
<td>To capture cells for counting or depletion</td>
<td>111.05/111.06</td>
<td>2ml/10ml vials</td>
</tr>
<tr>
<td>Dynabeads® CD8</td>
<td>To capture cells for counting or depletion</td>
<td>111.07/111.08</td>
<td>2ml/10ml vials</td>
</tr>
<tr>
<td>Dynabeads® CD14</td>
<td>To deplete monocytes</td>
<td>111.11/111.12</td>
<td>2ml/10ml vials</td>
</tr>
<tr>
<td>Dynabeads® CD19</td>
<td>To deplete B cells</td>
<td>111.03/111.04</td>
<td>2ml/10ml vials</td>
</tr>
<tr>
<td>Dynabeads® Pan Mouse IgG</td>
<td>To couple with any subclasses of mouse IgG for multiple depletions</td>
<td>110.22/110.23/110.24</td>
<td>2ml/10ml vials</td>
</tr>
<tr>
<td>Dynabeads® M-450 Rat anti-Mouse IgM</td>
<td>To couple with mouse IgM for 5ml-50ml samples. Holds six microtubes.</td>
<td>112.01</td>
<td>2ml vials</td>
</tr>
<tr>
<td>Dynal® MPC®-1</td>
<td>For 20µl-2ml samples. Holds six microtubes.</td>
<td>120.20</td>
<td>1 unit</td>
</tr>
<tr>
<td>Dynal® MPC®-S</td>
<td>For 5ml-50ml samples. Holds six blood tubes.</td>
<td>120.21</td>
<td>1 unit</td>
</tr>
</tbody>
</table>