Human Dendritic Cells

Antigen presenting cells - key cell type in the human immune system

Dynabeads® can be used to provide a large source of dendritic cells for studies

CD₃₄ derived dendritic cells - CD₃₄⁺ cells can be cultured in the presence of cytokines to develop into CD₃₄ derived dendritic cells 1, 2).

Monocyte derived dendritic cells - monocytes can be cultured in the presence of cytokines to develop into monocyte derived dendritic cells 3, 4).

Dendritic cells (DC) (fig. 1) are important antigen presenting cells. DC are mononuclear phagocytic cells which uptake antigens by endocytosis and then present antigen peptide in their MHC class II molecule to T cells. DC also express the B7 co-stimulatory molecule. The cells do not require activation prior to functioning as an antigen presenting cell (APC). DC can activate naive T cells, effector T cells and memory T cells.

There are several sources of human dendritic cells. Blood dendritic cells account for approximately 0.1% of leucocytes. They express cell surface antigens such as CD1a. Other circulating DC are found in the lymph nodes (veiled cells). Interdigitating DC are found in lymphoid organs. DC are also found in non-lymphoid organs such as the skin (Langerhans cells) and heart, lungs and gastrointestinal tract (interstitial DC). All these DC express MHC class II for antigen presentation to T cells. Follicular DC however do not express MHC Class II and appear to have a different origin and function to the DC described above.

As DC are potent APC and are key cells in the adaptive immune system, they are frequently studied. However, as blood DC are so rare, it is common to derive DC for studies. DC can be generated from blood monocytes or from CD₃₄⁺ progenitor cells. Dynal Biotech provides systems for the isolation of both these cell types for subsequent generation of large numbers of dendritic cells.

Dendritic cells from CD₃₄⁺ progenitor cells

- Positive isolation of CD₃₄⁺ cells from bone marrow is simple and fast (90 minutes).
- Culturing of CD₃₄⁺ cells with GM-CSF and TNFα for 4-5 days and GM-CSF, IL-4 and TNFα for a further 9 days induces DC phenotype.
- Cultured DC can present antigens to T cells.

CD₃₄⁺ cells can be simply and effectively isolated from bone marrow using the Dynal® CD₃₄ Progenitor Cell Selection System. This kit contains Dynabeads® for the positive isolation of the CD₃₄⁺ cells plus DETACHaBEAD® for the release of these captured cells from the beads. Purity of these cells is 95% and they show excellent viability. The cells can then be cultured in RPMI / 10% FCS with GM-CSF (250 ng/ml) and TNF-α (50 ng/ml) and optional stem cell factor (50 ng/ml) for 4-5 days (changing medium every 3 days).

They are further cultured with GM-CSF (250 ng/ml), IL-4 (100 ng/ml) and TNF-α (50 ng/ml) for 9 days. During this period DC phenotypic markers are expressed (CD1a, CD4, CD11c, CD40, CD86, HLA-DR), and CD14 down-regulated. The resulting DC are capable of presenting antigens to T cells.

Fig. 1: Human dendritic cells

Fig. 2: Surface phenotype of monocyte derived dendritic cells after culture. The histograms show fluorescence value on gated large cells.
Dendritic cells from peripheral blood monocytes

- Negative isolation of monocytes from peripheral blood MNC is simple and fast (45 minutes).
- Culturing of monocytes with IL-4 and GM-CSF induces DC phenotype after 7 days.
- Cultured DC can present antigens to T cells.

Monocytes can be negatively isolated from peripheral blood MNC by removing T cells, B cells, NK cells and granulocytes (if present) using the Dynal® Monocyte Negative Isolation Kit (Fig. 3). Negatively isolated monocytes are pure and viable and can be cultured in RPMI / 10% FCS with IL4 (100 ng/ml) and GM-CSF (250 ng/ml) for 7 days (changing medium every 3 days). During this period DC phenotypic markers are expressed (CD1a, CD4, CD11c, CD40, CD86, HLA-DR), and CD14 down-regulated (fig. 2). DC can be tested for antigen-presenting capacity in a mixed lymphocyte reaction with negatively isolated T cells and protein (PPD) (Fig. 4). Results show that Dynabeads® can be used as an efficient tool for obtaining functional DC derived from peripheral blood monocytes and the DC are capable of presenting antigens to T cells.

Fig. 3: Overview of negative isolation of monocytes and generation of dendritic cells.

Fig. 4: Cultured DC present PPD efficiently to T cells. DC were used as APC for negatively isolated T cells. 5 x 10^4 T cells were stimulated with different numbers of DC or irradiated MNC (3000 rad) for 7 days in the presence of 2 mg/ml of PPD. Proliferation was measured by 3H-thymidine incorporation.

### References


### Ordering Information

<table>
<thead>
<tr>
<th>Dynal Product</th>
<th>Prod. No.</th>
<th>Kit Components</th>
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<tbody>
<tr>
<td>Dynal® CD34 Progenitor Cell Selection System</td>
<td>113.01</td>
<td>Dynabeads® CD34 &amp; DETACHaBEAD®, 5 ml kit</td>
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<tr>
<td></td>
<td></td>
<td>1 ml binds 8 x 10^7 CD34+ cells</td>
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<tr>
<td>Dynal® Monocyte Negative Isolation Kit</td>
<td>113.09</td>
<td>Depletion Dynabeads®, Antibody Mix &amp; Blocking Agent Kit isolates &gt; 1 x 10^8 monocytes</td>
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For all Dynabeads® applications, a magnetic rack (Dynal MPC®) is required. For full details of all Dynal MPC®, please see Dynal Biotech Immunosystems Manual, www.dynalbiotech.com or contact your local supplier.

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