Dendritic cells are key regulators of both the innate and adaptive immunity. The majority of dendritic cells are called ‘conventional’ dendritic cells (cDCs) and defined as ‘antigen-presenting-cells’ (APC), due to their primary function in recognizing pathogens, processing antigens and presenting them to T cells which initiates adaptive immune responses. The ability of cDCs to regulate immunity is dependent on their maturation status. In response to activation during infection, injury or vaccination, cDC undergo maturation enabling them to activate clonal naïve T cell expansion and T effector cell differentiation. cDCs maturation is characterized by a variety of phenotypical and functional changes; these include the relocation of the major histocompatibility complex (MHC) molecules to the cell surface, the up-regulation of cell surface molecules including CD86, CD80 and CD40, the formation of dendrites, and secretion of cytokines such as IL-12-7. One mechanism which mediates this cDC maturation is the binding of CD40 ligand (CD40L) to its receptor. CD40/CD40L interaction leads to the activation of p38 MAP Kinase (MAPK) and Jun Kinase (JNK) pathways and up-regulation of CD83, CD80, CD86 and MHC molecules, which results in T cell activation. cDCs are therefore considered key regulators for the interplay between innate and adaptive immune responses. As a consequence, deregulation or modulation of cDC functions are crucial events in the development of adaptive immune responses and they have been implicated in several diseases such as HIV infection, allergy, rheumatoid arthritis, autoimmune encephalomyelitis, and cancer. Hence, evaluating compound effects on cDCs maturation and activation offers a unique approach for the identification of drug candidates for these disease, as well as for DCs-mediated cellular therapies. Identified cytokines can be future studied in vivo across an immunology platform.
Dendritic cells can be differentiated from blood-derived monocytes and from umbilical cord blood CD34+ hematopoietic progenitor cells, or they can also be directly isolated from dermal or mucosal biopsies. While the last two approaches can be extremely laborious with multiple handling steps resulting in an insufficient number of cells, the differentiation of dendritic cells from blood monocytes has shown to be more a more efficient and reliable method resulting in higher cell yield, lower handling steps, and strong reproducibility across multiple donors. Because of their straightforward differentiation protocol and their physiological and morphological similarities to cDCs, monocyte blood-derived dendritic cells are considered the ideal source to assess in vitro cDC functions for drug development studies. We have therefore developed a validated cDCs activation assay using primary human monocytes freshly isolated from blood of healthy donors to study the effects of compounds on cDCs functions.

**Assay Principle**

Human monocytes are purified from peripheral blood mononuclear cells (PBMCs) using an anti-CD14 antibody coupled to magnetic beads (positive selection approach). CD14+ isolated cells are then cultured in the presence of interleukin 4 (IL-4) and granulocyte macrophage-colony-stimulating factor (GM-CSF) which drive the differentiation of monocytes into cDCs. After 5 days, the cDCs are seeded in a 96-well plate. The trigger, sCD40L (soluble CD40L), is added for 24h to induce their activation and maturation, while potential drug candidates for compound testing are added simultaneously. cDCs are analyzed for their maturation signature (CD40+HLA-DR+CD11c+CD83int/CD14low) by flow cytometry and for their activation by measuring IL-12p40 secretion using the MSD platform.

**Assay Workflow**

- **D0**
  - Isolate CD14+ cells from buffy-coats
  - Add IL-4 and GM-CSF
  - Cell QC by flow cytometry

- **D3**
  - Refresh media

- **D5**
  - Seed cells
  - Add compounds, controls, and trigger

- **D6**
  - Cell QC by flow cytometry

Harvest supernatant (IL12p40 MSD)

**Complex Biology In Vitro Assays: Immunology**
Assay Setup

<table>
<thead>
<tr>
<th>Cells</th>
<th>CD14^+ cells (monocytes) from healthy human blood donors</th>
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</thead>
<tbody>
<tr>
<td>Differentiation factors</td>
<td>IL-4 [20 ng/mL], GM-CSF [20 ng/mL] for 5 days</td>
</tr>
<tr>
<td>Seeding density</td>
<td>1x10^6 cells/well in 96-well plate</td>
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<tr>
<td>Maturation trigger</td>
<td>sCD40L [500 ng/ml] for 1 day</td>
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<tr>
<td>Inhibitor</td>
<td>Dexamethasone at 8 point concentration response curves (CRC)</td>
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<tr>
<td>Compounds</td>
<td>8-point concentration response curves (in biological duplicates)</td>
</tr>
<tr>
<td>Controls</td>
<td>Complete medium (RPMI 1640, 10% FBS, 1% Pen/Strep)</td>
</tr>
<tr>
<td></td>
<td>Vehicle: DMSO (0.1 %)</td>
</tr>
<tr>
<td>Time point assay</td>
<td>24 hours (post-trigger/compound addition)</td>
</tr>
<tr>
<td>Readout</td>
<td>IL-12p40 secretion (MSD)</td>
</tr>
</tbody>
</table>

Quality Checks

Flow cytometry is used to perform a quality check (QC) of freshly isolated monocytes to use in the assay. Isolated monocytes are stained with a specific monocyte marker (anti-CD14 antibody) and its relative isotype control, and analyzed by flow cytometry (Fig. 1). Donors that exhibit > 80% of CD14 expression are used for the cDCs activation assay.

A second QC is performed 24h after trigger addition to assess the cDCs maturation status. Cells are stained with anti-CD40, anti-HLA-DR, anti-CD11c, anti-CD83 and anti-CD14 antibodies and their relative isotype controls, and analyzed by flow cytometry (Fig. 2A). The majority of donors tested exhibited a consistent expression of cDC maturation markers (Fig. 2B) according to the expected cell phenotype.

![Figure 1. Quality check of blood-derived monocytes (CD14^+) isolated cells. Representative flow cytometry histograms show 96% of CD14 expression on monocytes isolated from one healthy blood donor. Isotype control shows no non-specific staining.](image)

![Figure 2. Quality check of cDCs maturation](image)

A. Representative flow cytometry histograms show the expression of cDCs maturation markers 24h after trigger addition from one donor.
B. Proportions of cells that expressed cDCs maturation markers (HLA-DR, CD40, CD11c, CD83) are shown for 4 representative human blood donors.
Assay Performance

Performance of the developed assay is validated using sCD40L as a trigger and dexamethasone as positive control for inhibition of cDCs activation, together with other assay controls. Data obtained from one monocyte-derived cDCs donor are reported below (Fig. 3).

Here we demonstrated an optimized cDC activation assay using monocyte-derived dendritic cells obtained from healthy blood donors using sCD40L at 500 ng/mL as main trigger for cDC activation and subsequent maturation. The addition of sCD40L to immature monocyte-derived cDCs induced the expression of maturation markers on the cell surface and showed increased levels of IL-12p40 cytokine, which was strongly inhibited by the addition of dexamethasone in a dose-dependent manner. IC50 values were consistent across different donors (not shown). Using this immunology assay, maturation of cDCs can be monitored to evaluate therapeutic candidates for the treatment of infections, and autoimmune diseases as well as cancer.

Therapeutic candidates can be evaluated in an 8-point CRC on cDCs derived from up to two healthy donors in biological duplicates for their capacity to release IL-12p40.
**Summary**

cDCs are key immune cells for the initiation and coordination of antigen-specific immune responses. The differentiation and maturation of cDCs are crucial processes for antigen presentation and T cell activation, characterized by increased expression of MHC and co-stimulatory molecules, and the release of cytokines such as IL-12p40. Upregulation of CD40 on the cDC surface and its interaction with CD40L activates a signaling cascade which is important for the initiation of adaptive immune responses.

Impaired ability of cDCs to prime T cells can affect the initiation of adaptive immune responses and have detrimental effects in the development of several pathological settings including infections, autoimmune diseases and cancer. Hence, understanding the mechanisms underlying cDCs maturation and finding new strategies that could lead to novel treatments or improve existing therapeutics.

For our clients’ scheduling convenience, our DCs activation assay will be performed on a monthly basis upon receipt of compounds. Results are issued within 4 weeks following compound receipt.

**Assay Reference Code**
cDC Activation Assay – Reference code

**OTS-219 cDC Activation Assay**

**Complementary Immunology Assays**

Fibroblast-like Synoviocyte Activation Assay

Chemotaxis Assay: Monocytes

Chemotaxis Assay: Neutrophils

**References**