Complex Biology *In Vitro* Assays: Immuno-Oncology Cytokine Response Assay

**TCR-Dependent Stimulation of Th-1, Th-2 and Th-17 Cytokine Biosynthesis in Isolated Human PBMCs**

The immune system has a very important role in tumor progression; hence, it is crucial to characterize the possible impact of a drug candidates (antibody or small molecule therapeutic) on the immune system. Our immune cell activation assays assess the impact of candidate compounds on multiple super antigen stimulated T-cell receptor (TCR) engagement pathways. The immuno-oncology assays are optimized for multiplex cytokine analysis and identified cytokines can be future studied *in vivo* across an immunology platform.

**Assay Principle**

Freshly isolated human PBMCs from healthy donors are seeded in the absence or presence of various stimuli (plate-bound anti-CD3, soluble anti-CD28, or PHA) for the indicated time (48 hours) in the presence or absence of test compounds. After 2 days, the cell culture supernatant is removed for multiplex cytokine analysis.

---

Need a custom version of this assay?
Assay Setup

T cell-activated protocol has been developed for optimum analysis of Th-1/Th-2/Th-17 cytokine response.

<table>
<thead>
<tr>
<th><strong>T Cell Activation Assay</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Donor</strong></td>
</tr>
<tr>
<td><strong>Seeding density</strong></td>
</tr>
<tr>
<td><strong>Trigger (positive control)</strong></td>
</tr>
<tr>
<td><strong>Incubation</strong></td>
</tr>
<tr>
<td><strong>Readout</strong></td>
</tr>
</tbody>
</table>

Representative dose response data shown below from one donor with multiple stimulants measured at 48 hours post-stimulation. The cytokines have been chosen to represent Th-1 (IFN-γ and IL-2), Th-2 (IL-5, IL-10 and IL-13) and Th-17 (IL-17) responses. Where both anti-CD3 and anti-CD28 stimulation were performed, a dose response to anti-CD3 was tested in the presence of a fixed concentration of anti-CD28 (10 μg/mL), except the graph for IL-2, in which case 5 μg/mL of anti-CD28 was used.

Cytokine Response Assay

- **IL-17 48 Hours**
- **IL-10 48 Hours**
- **IFN-γ 48 Hours**
- **IL-5 48 Hours**
- **IL-13 48 Hours**
- **IL-2 48 Hours**
**Summary**

Type 1 T helper (Th-1) cells produce proinflammatory cytokines interferon-gamma, interleukin (IL)-2, and tumor necrosis factor (TNF)-beta, which activate macrophages and are responsible for cell-mediated immunity and phagocyte-dependent protective responses. By contrast, type 2 T helper (Th-2) cells produce IL-4, IL-5, IL-10, and IL-13, which are responsible for strong antibody production, eosinophil activation, and inhibition of several macrophage functions, thus providing phagocyte-independent protective responses. Th-2 responses will counteract the Th-1 mediated action.

Th-17 cells are another T helper subset that are characterized as preferential producers of interleukin-17A (IL-17A), IL-17F, IL-21, and IL-22. Th-17 cells and their effector cytokines mediate host defensive mechanisms to various infections, especially extracellular bacterial infections, and are involved in the pathogenesis of many autoimmune diseases. The optimal scenario would therefore be that there should be a well-balanced Th-1, Th-2 and Th-17 response, suited to the specific immune challenge.

Here we show robust dose responses from multiple donors evaluating key cytokines that are involved in these T helper cell cytokine responses, Th-1 (IFN-γ and IL-2), Th-2 (IL-5, IL-10 and IL-13) and Th-17 (IL-17) using three different stimuli exploring T cell activation pathways.

Using these immuno-oncology assays with specified stimuli of interest, changes in immunoreactivity of molecules can be identified in isolated primary human PBMCs from our in-house donor panel. A single cytokine or a panel can be chosen from the examples shown to evaluate immunoreactivity of molecules.

**Assay Reference Codes**

- T cell CD3 Activated Th-1/Th-2/Th-17 Cytokine Response Assay
  
  Assay reference code: OTS106-TCELLCR-Th1Th2Th17-CD3

- T cell CD3 + CD28 Activated Th-1/Th-2/Th-17 Cytokine Response Assay
  
  Assay reference code: OTS107-TCELLCR-Th1Th2Th17-CD3CD28

- T cell PHA Activated Th-1/Th-2/Th-17 Cytokine Response Assay
  
  Assay reference code: OTS108-TCELLCR-Th1Th2Th17-PHA

**Complementary Immuno-Oncology Assays**

- T Cell Proliferation CTG Assay
- T Cell Exhaustion Assay
- T Cell-Mediated Chemotaxis Assay
- 3D Spheroid T Cell Cytotoxicity Assay