Lophius Biosciences GmbH
Experts for immune diagnostic systems

Lophius’ developments are based on its expertise in cell-mediated immunity (CMI) as well as on its proprietary T-activation® and Reverse T Cell Technology platforms. With its T-Track® CMV leading product, Lophius offers a highly sensitive, reliable and standardized CE-marked in vitro diagnostic solution to measure the functionality of CMV-specific CMI. T-Track® CMV assists clinicians in the risk stratification of CMV disease in immunocompromised patients, toward an improved and individualized patient management.

### T-Track® CMV

The CE-marked in vitro diagnostic test is a highly-standardized ELISpot assay. T-Track® CMV enables highly-sensitive detection of CMV-specific effector cells for:

- Assessment of CMV-specific CMI in healthy and immunosuppressed individuals
- Measurement of IFN-γ-producing effector cell reactivity against CMV antigens
- Assistance in antiviral therapy decision-making

### T-activated® proteins

T-activated® proteins enhance the stimulatory capacities of proteins by mimicking a natural infection.

- Proteins modified by Lophius’ T-activation® Technology
- Activate clinically-relevant populations of effector cells including CD4+ and CD8+ T cells as well as NK and NKT-like cells
- Applicable for many immunological assays such as ELISpot, FACS, ELISA

### Technology platforms

Lophius patented technologies enable the development of T cell-based diagnostic solutions for multiple indications with maximum flexibility in terms of read-out systems. Contact us or visit our website to learn more about:

- T-activation® Technology
- Reverse T Cell Technology
ELISPot
Tracking cell responses.
ELISPOT TECHNOLOGY

The Enzyme-Linked Immunosorbent Spot (ELISpot) is a common method for monitoring cellular immune responses to antigen stimulation. The general setup consists of a 96-well microtiter plate (strip or solid format), with a PVDF membrane at the bottom of each well. A capture antibody is immobilized onto the membrane to bind cytokines secreted by the antigen-stimulated cells. After applying an enzyme-conjugated detection antibody and following an enzymatic staining reaction, spots are revealed whereby one spot represents the unique cellular activity of antigen-specific cytokine-secreting cells at a single-cell level.

Membrane coating with capture antibodies
Capture antibodies (yellow mAb) specific for the cytokine of interest are immobilized on the PVDF membrane at the bottom of each well (grey well).

Cell stimulation followed by cytokine secretion
A defined number of isolated cells (e.g. PBMCs, grey cell) is added to each well and incubated with selected stimulants (red antigen). This leads to the secretion of cytokines (dark red dots) by antigen-specific reactive cells.

Cytokines bound by capture antibodies
Secreted cytokines are immediately captured by specific antibodies. Cells are eventually removed and membranes are washed several times to eliminate unbound cytokines.

Binding of detection antibodies
A specific detection antibody (red mAb) is added, either directly conjugated to an enzyme (one-step detection, as shown in the picture, green dot) or biotinylated, requiring the addition of a biotin-specific enzyme-conjugated polypeptide (two-step detection, not shown).

Enzymatic precipitation of substrate
A soluble substrate (A) is added to each well. The substrate is then converted to a colored precipitating product (B) by the Ab-conjugated enzyme (green dot). Thereby, visible spots are generated on the surface of the membrane, each representing the release of cytokines by one single cell.

Read-out of spots
A calibrated ELISpot reader or a microscope is used to quantify the spots (grey dots). According to this read-out step, the number of cytokine-secreting cells is calculated.
ELISPOT ADVANTAGES

The ELISpot assay allows to determine the frequency of cytokine-secreting cells at a single-cell level. It therefore addresses the functionality of antigen-specific reactive cells. Additionally, this technology is one of the most sensitive, specific and reproducible techniques available today to quantify particularly low frequencies of cellular activity, roughly up to one in a million cells. Advantages of ELISpot in comparison to other methodologies are displayed in the table below.

<table>
<thead>
<tr>
<th>Advantage</th>
<th>ELISA</th>
<th>ELISPOT</th>
<th>FACS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of cytokine-secreting cells</td>
<td>✔</td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>Total amount of secreted cytokine</td>
<td>✔</td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>High standardization</td>
<td>✔</td>
<td>✔</td>
<td></td>
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<tr>
<td>Single-cell level analysis</td>
<td>✔</td>
<td>✔</td>
<td></td>
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<tr>
<td>Sorting of cells based on physical properties</td>
<td>✔</td>
<td>✔</td>
<td></td>
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<tr>
<td>Donor to donor variation</td>
<td>✔</td>
<td>✔</td>
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Immune monitoring ELISpot kit: T-Track® CMV

Current immune monitoring assays for e.g. cytomegalovirus (CMV) are based on MHC multimer staining (by FACS), intracellular staining of cytokines (by FACS), and interferon-gamma (IFN-γ) release assays (IGRAs) combined with an ELISA or ELISpot read-out.

Most of these assays have limitations in terms of sensitivity (ELISA), ability to measure cell functionality (Multimer staining) and standardization capability (ICS-FACS). T cell-based IFN-γ ELISpot assays rely on the detection of IFN-γ-secreting CMV-reactive effector cells after stimulation of whole blood or peripheral blood mononuclear cells (PBMC) with CMV-specific antigens or peptides. It is as yet the most sensitive assay, measures CMV-specific cell functionality at a single-cell level and presents the potentiality for standardization. One outstanding advantage of the CE-marked in vitro diagnostic IFN-γ ELISpot test T-Track® CMV is to measure the functionality of a broad range of CMV-specific effector cells, including CD8+ T cells (cytotoxic T cells or CTL), CD4+ T cells (T helper or Th), natural killer (NK) cells and NKT cells.