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# Immuno-Oncology *In Vitro* Assays

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## Abstract

We offer a wide range of assay types and formats to characterize immune-oncology monoclonal antibodies (mAbs). In this application note, we discuss our recently developed assay format to characterize checkpoint inhibitor mAbs, specifically discussing those that target the PD-1 PD-L1 pathway.

Targeting immune checkpoints such as the PD-1 PD-L1 pathway has proven in recent years to be an effective treatment approach resulting in a range of approved mAbs that bind PD-1 or its ligand PD-L1. The checkpoint inhibitor pathways continue to be an area of intensive research and development to bring other Mab treatments to the market. Characterizing the mechanism of action (MoA) of these molecules requires complex assays using primary cells because the MoA rely upon the interaction between T cells and antigen presenting cells.

Utilizing our assay development expertise, the high through-put iQue Screener PLUS flow cytometer newly manufactured by Sartorius and long-established ELISA approaches, we developed a flexible mixed Lymphocyte Reaction (MLR) assay format, allowing us to work with our clients to characterize their molecule of interest in a MLR assay. Depending on the client's requirements, the flexible assay format can have multiplexed flow cytometry or ELISA endpoints to characterize the molecule of interest.

**Find out more:** [www.sartorius.com](http://www.sartorius.com)

## Mixed Lymphocyte Reaction

Immunotherapy harnesses the immune system to fight cancer. Recent research has focused on teasing tumor cells out from their protective micro-environments by exposing them to the patient's immune response, leading to the development of new immunotherapeutic drugs. Immune checkpoints are the regulators of this balancing act and have become a key target for oncology therapeutics. Binding T-cell-expressed inhibitory checkpoints such as PD-1 and CTLA-4 with their respective ligands inhibit cell activation. Checkpoint inhibitors can block this signaling cascade, keeping T cells in their active state (see Figure 1) and effectively taking the brakes off cancer-fighting immune cells.

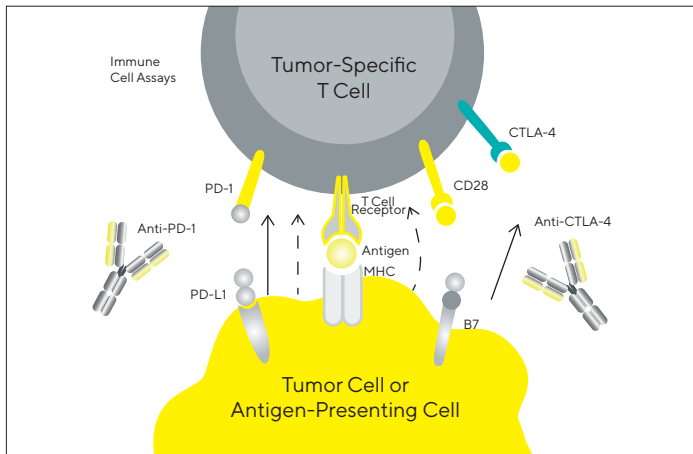


Figure 1. An immune synapse formed between an antigen-presenting (APC) or tumor cell and a tumor-specific T cell. The illustration highlights the interactions targeted by immune checkpoint therapeutics such as anti-PD1 and anti-CTLA-4.

Evaluating immune checkpoint drug candidates' functionality *in vitro*, requires a cell-based immuno-assay. Our mixed lymphocyte reaction assay (MLR), which measures T cell activation in response to mismatched major histocompatibility complex (MHC) antigens can be adopted for this purpose.

## Assay Outline

CD4+ T cells are incubated together with allogeneic, activated, mature Dendritic cells (mDC) in the presence or absence of test compounds. Donor pairs can be used repeatedly, or numerous donor pairings can be used. All donors are HLA typed.

After assay incubation, a sample of cells and/or supernatant is removed to measure T cell activation. There are several reporting options for this assay, including cytokine release, T cell proliferation and T cell activation markers (see Figure 2). Only small sample volumes are required for flow cytometry assays, so multiple time points can be analyzed from one assay plate.

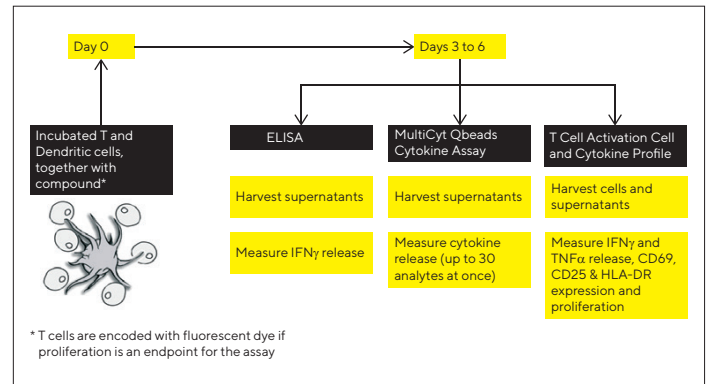


Figure 2. Diagram of assay design illustrating flexibility in data output from the mixed lymphocyte reaction according to client requirements.

## Cytokine Response using Flow Cytometry

Cytokine release can be reported using a cytokine bead assay, which has the benefits of requiring only small sample volumes and can read out multiple cytokines at one time. The example below (Figure 3), shows representative data for IFN $\gamma$  and TNF $\alpha$  from supernatants analyzed on day four. There is a titratable enhancement of IFN $\gamma$  and TNF $\alpha$  release by blocking PD-1 and PD-L1 using multiple checkpoint inhibitor drugs.

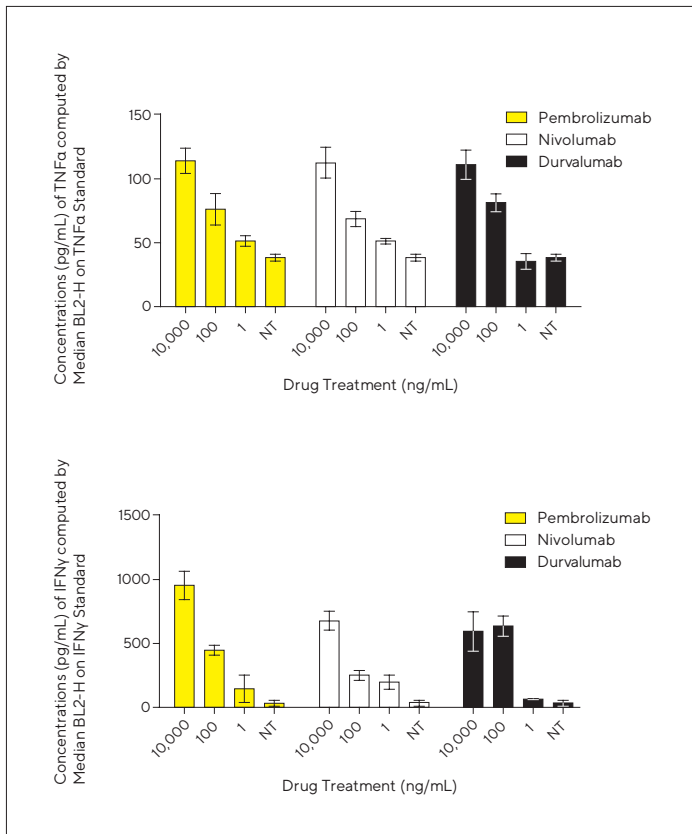


Figure 3. MLR induced cytokine release is increased in response to anti-PD-1 and PD-L1 treatment. CD4+ T cells were incubated with mDCs for four days with three concentrations of nivolumab, pembrolizumab or durvalumab. Cytokine release was measured using the Human T Cell Activation Kit on the iQue Screener PLUS. NT = T cells and mDCs with no molecule. The error bars (n=3) indicate mean ± SEM.

Due to the small sample volume required for this cytokine bead assay, cytokine release data can be collected at several time points from one assay plate to measure the immune response over time (Figure 4).

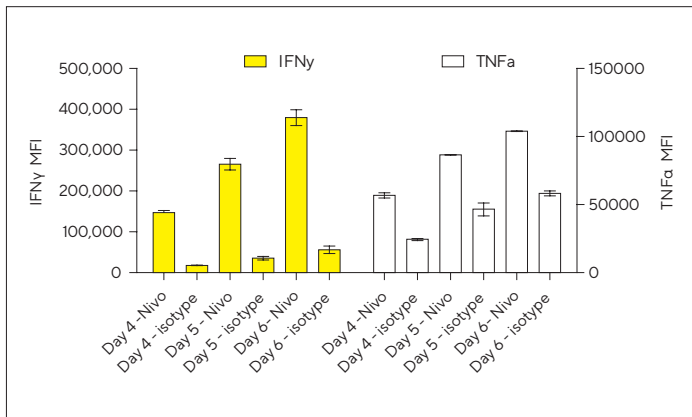


Figure 4. MLR induced cytokine release is increased over time in response to nivolumab treatment. CD4+ T cells were incubated with mDCs for four, five, and six days with 9000 ng/mL nivolumab or isotype control antibody. Cytokine release was measured using the Human T Cell Activation Kit on the iQue Screener PLUS. The error bars (n=3) indicate mean ± SEM.

## IFNγ Cytokine Response using ELISA

Cytokine production as an endpoint of the MLR assay can be measured by methods other than flow cytometry. An ELISA can be used to determine the concentration of cytokines such as IFNγ released in the MLR assay (Figure 5).

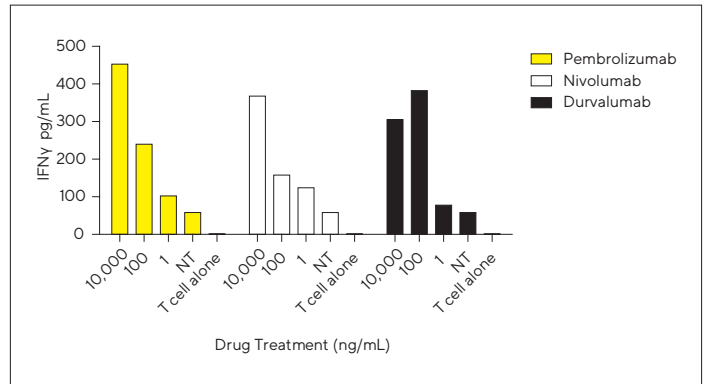


Figure 5. MLR induced IFNγ release is increased in response to anti-PD-1 and anti-PD-L1 treatment. CD4+ T cells were incubated with mDCs for four days with three concentrations of nivolumab, pembrolizumab or durvalumab. Cytokine release was measured using ELISA. NT = T cells and mDCs with no drug treatment. Concentration interpolated in Soft-Max from mean O.D. value from a known standard (n=3).

## Summary


An effective checkpoint inhibitor will enhance IFNγ cytokine release in an MLR assay, we can capture this biological action using traditional ELISA and/or multiplexed flow cytometry methods that can include the assessment of cell surface activation markers. The flexibility of the assay data output allows the client to choose the best endpoint to characterize their molecule's mechanism of action.

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