



Mixed Lymphocyte Reaction Assay (MLR)

MIXED LYMPHOCYTE REACTION (MLR)

Our MLR Assay allows for the rapid identification of agents that modulate T cell activation to assay biologics or small molecules as single agents or in combination *in vitro*. Leveraging our extensive experience in immunology and oncology, we provide analysis and interpretation of the effects of test agents on multiple endpoints using multiple allogeneic donor pairs (for example, human PBMC/CD4+ T/DC cells, mouse splenocytes, etc).

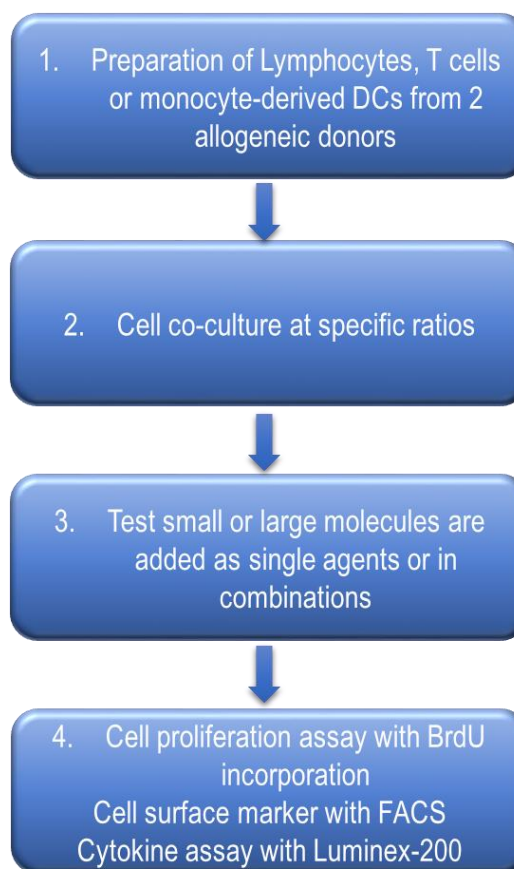
One-way MLR

One-way MLR is a lymphocyte proliferation assay, where responder lymphocytes (such as CD4+ T cells) from one donor are stimulated to proliferate by stimulator lymphocytes from another donor. The stimulator cells (such as allogeneic dendritic cells) are pre-treated with mitomycin-c. Lymphocytes are co-cultured for several days. Secreted cytokines (such as IL-2, IFN- γ) in cell culture are quantified. Bromo-deoxyuridine (BrdU) is added to cell culture to determine proliferating T cells.

Two-way MLR

A two-way MLR differs from a one-way MLR in that both donors of lymphocytes stimulate each other and are able to proliferate.

HOW DOES MLR ASSAY WORK?



OUR SERVICE FEATURES

- ❖ **High throughput:** 48~384-well assay
- ❖ **Robust and highly reproducible** assay suitable for small to large scale screening
- ❖ **Single agents or combinations:** Flexible assay design to fit specific project
- ❖ **Multiple allogeneic donors:** PBMC or enriched primary T cells with various genotypes (such as Fc γ RIIIa 158 V/V) and monocytes derived dendritic cells from multiple donors



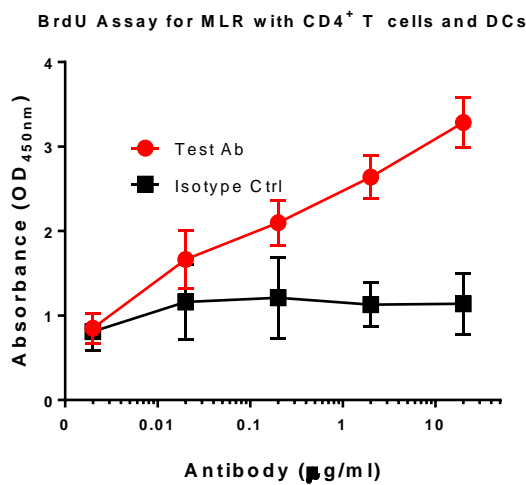
to address donor-to-donor variability

- ❖ **Multiple endpoints:** Surface markers (such as regulatory macrophages), cytokines (such as IL-2, IFN- γ), and cell proliferation -- clearly understand the immunomodulatory profile of test agents
- ❖ **State-of-art platforms:** **Luminex-200** for multiplex cytokine analysis; **CytoFLEX S** (Flow cytometer with 4

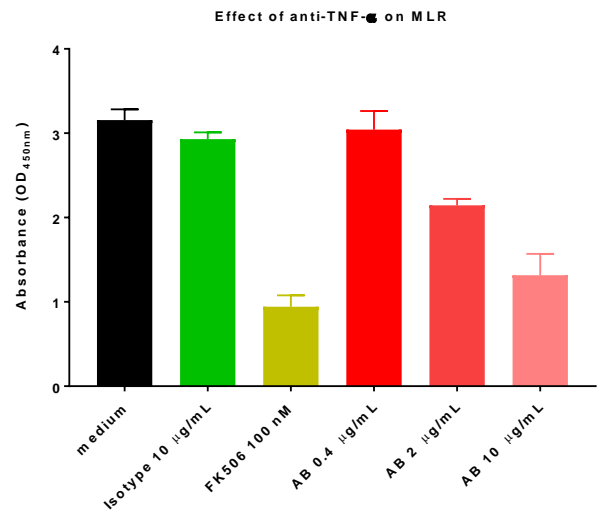
lasers 13 colors) for cell identification and quantification.

- ❖ Fully validated and quality-controlled
- ❖ Multiple concentrations in triplicate
- ❖ Positive and negative controls: isotype Ig G, inhibitor and stimulator controls
- ❖ Quick turn-around time
- ❖ 20+ years of hands-on experience: Expert data analysis and interpretation, scientific and technical support

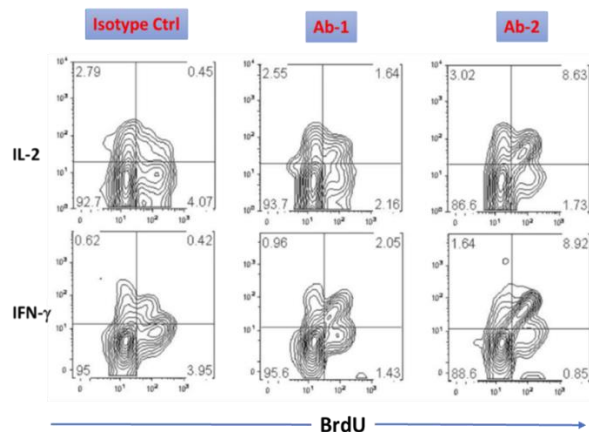
A)



C)



B)



Examples. A) BrdU incorporation in a mixed lymphocyte reaction of human monocytes-derived dendric cells and CD4⁺ T cells in the presence of increasing concentrations of anti-PD-1 and isotype control antibody.

B) IL-2 and IFN- γ production in proliferating T cells in MLR in the presence of anti-PD1 antibodies was analyzed by flow cytometry.

C) BrdU incorporation in a mixed lymphocyte reaction of 2 allogeneic donors of human PBMCs in the presence of increasing concentrations of anti-TNF- α , FK506, and isotype control antibody.