



# MACROPHAGE CELL-BASED ASSAYS

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- Macrophage Polarization Assay
- Phagocytosis Assay
- Monocyte Killing Assay
- Multiplex Cytokine Assay



Macrophages, integral to the innate immune system, specialize in phagocytosis of pathogens and diseased cells, as well as secreting pro-inflammatory agents. In cancer and chronic inflammation macrophages often gain an anti-inflammatory phenotype and contribute to cancer progression.

Macrophages are characterized by high plasticity, and it has been shown that their polarization state and function can be modulated therapeutically. Therefore, macrophage targeting therapies are considered as next generation cancer immunotherapies.

At Reaction Biology, our scientists have devised a comprehensive array of *in vitro* assays tailored to studying macrophage function, specifically focusing on their involvement in the tumor microenvironment.



### Macrophage Polarization Assay

**Format: Flow cytometry**  
*Phenotype via surface marker & cytokine release*



### Phagocytosis Assay

**Format: Flow cytometry**  
*Analysis of cellular response & live-cell imaging using labeled bioparticles*



### Monocyte Killing Assay

**Format: Plate based detection**  
*Quantifying tumor cell viability through luciferase expression*

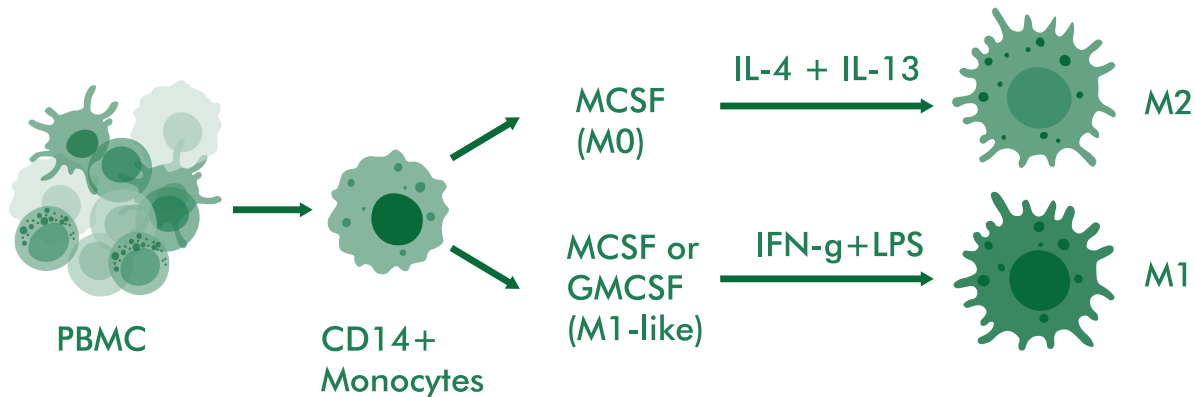


### Multiplex Cytokine Assay

**Format: Sandwich ELISA**  
*Quantifying cytokine response*

# Macrophage Polarization Assay

Test the effect of your immunotherapeutic compound on macrophage differentiation, within the tumor microenvironment or in response to inflammation and autoimmunity.

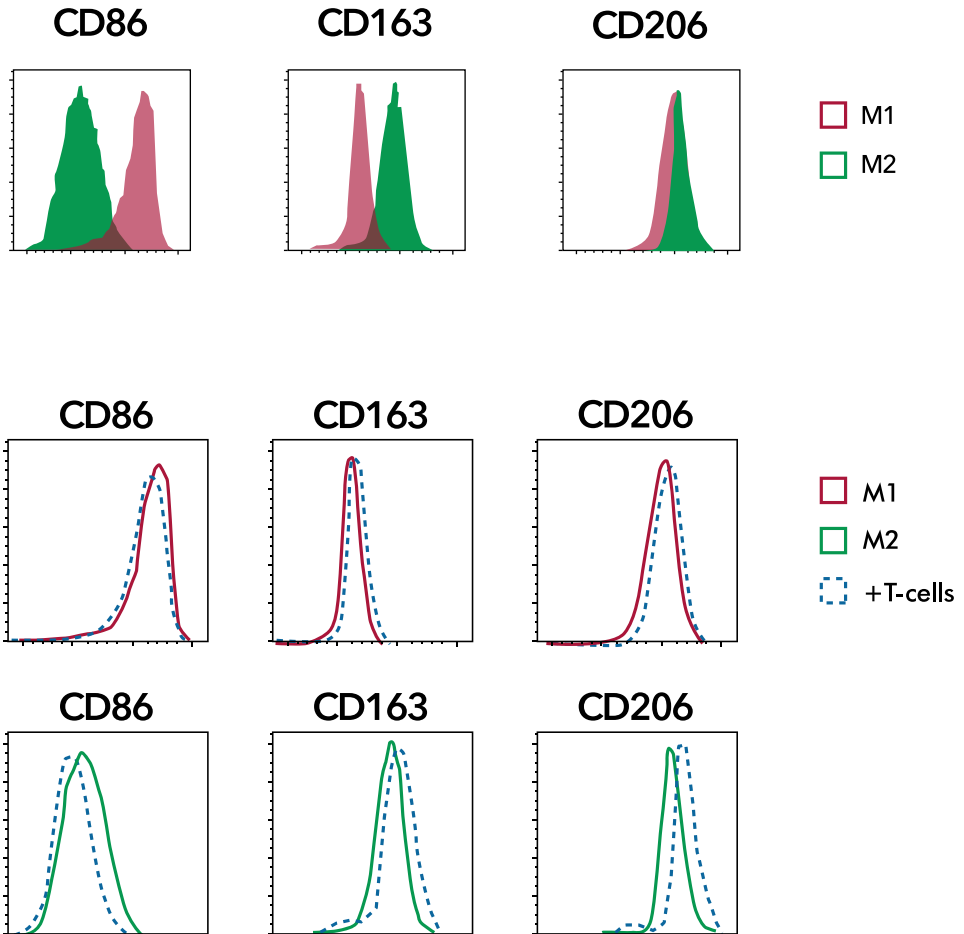


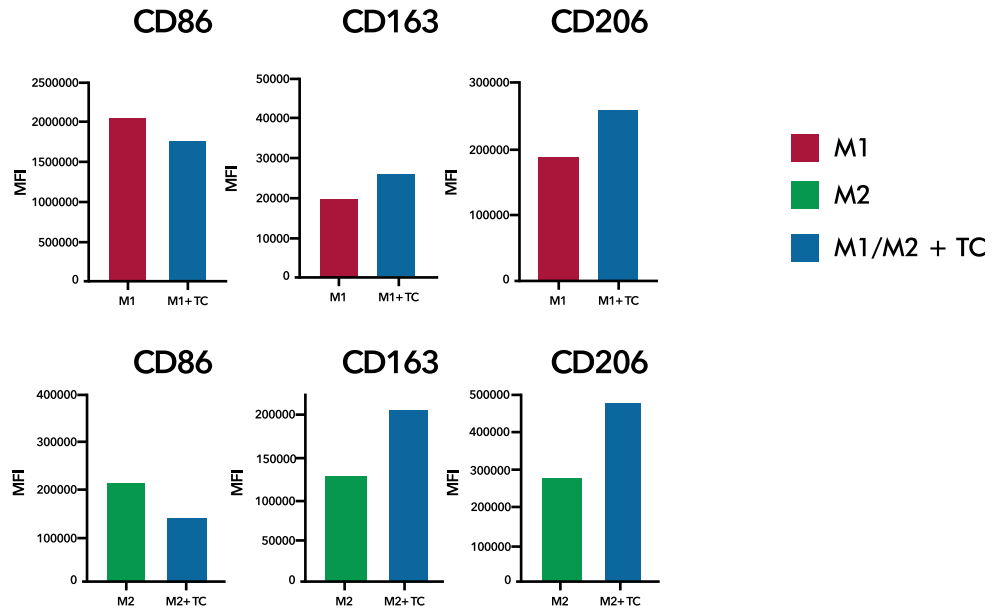
*Workflow depicting the isolation of CD14+ monocytes from donor PBMCs and their polarization into M1 and M2 macrophages*

## Experimental workflow

- Isolation of CD14+ monocytes from healthy donor PBMCs.
- Culturing cells under specific conditions to promote polarization into:
  - Pro-inflammatory M1 macrophages
  - Anti-inflammatory M2 macrophages

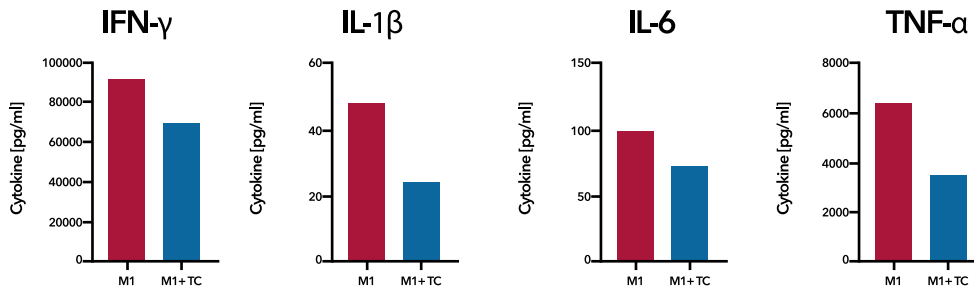
- Verification of the differentiation process through flow cytometry, identifying key surface markers such as CD86, CD163, and CD206 to distinguish macrophage phenotypes





*Assessment of macrophage differentiation into specific phenotypes through the analysis of M1/M2 phenotype markers using flow cytometry. TC=Test compound*

- Combine cell phenotype with functional assays and cytokine measurements indicative of M1 and M2 types



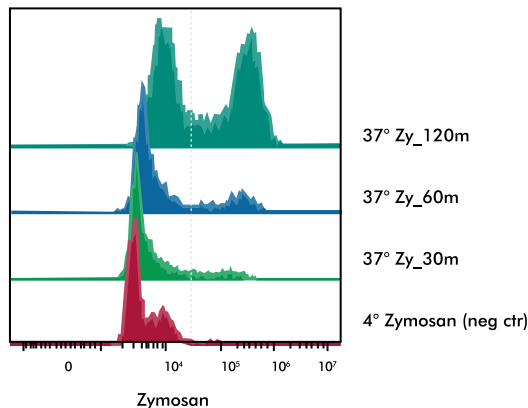
*Multiplex cytokine analysis to assess signature phenotype of macrophage differentiation and polarization*

# Phagocytosis Assay

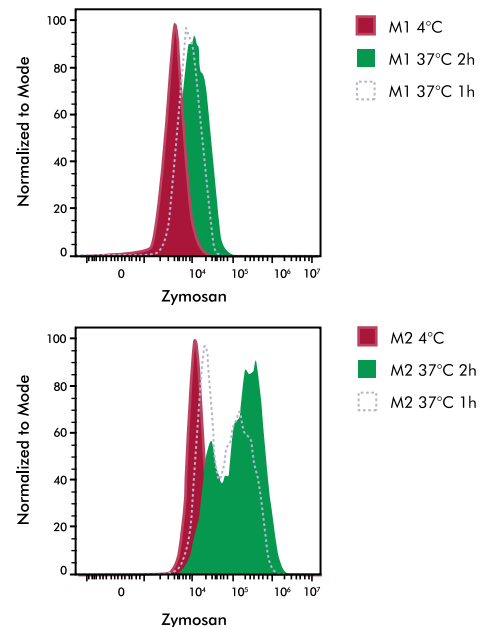
Assess the phagocytic capabilities of various monocyte/macrophage subtypes utilizing labeled bioparticles

## Experimental workflow

- Use of fluorescently tagged bioparticles, such as Zymosan-pHRodo and Ecoli-pHRodo
- Assessment of phagocytic activity by measuring internalization of flow cytometry analysis or real-time measurements using microscopy
- Comparing phagocytic capacity of various macrophage types or macrophage treatments



Representative histograms depicting monocyte (left) and polarized macrophage (right) phagocytosis using zymosan reporter particles as measured by flow cytometry

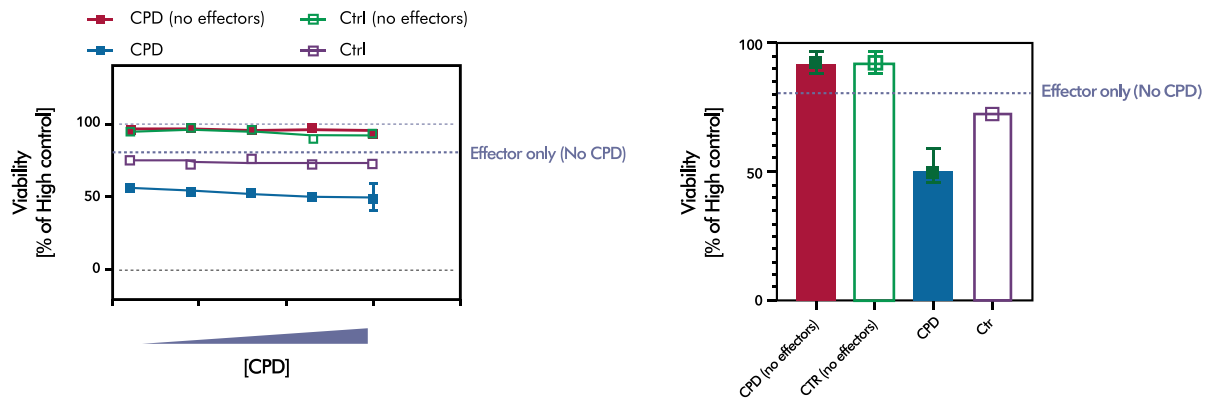


# Monocyte Killing Assay

Investigate the efficacy of biologics by measuring their ability to kill target cells and induce monocytes to release inflammatory cytokines

## Experimental workflow

- Isolation of CD14+ monocytes from PBMCs
- Co-culturing with target cells that express luciferase, at 10:1 (E:T ratio) effector to target ratio, meaning ten monocytes for every target cell
- Assessment of luciferase expression as a readout of viability of target cells
- Optional: detection of inflammatory cytokine secretion in cell culture supernatants



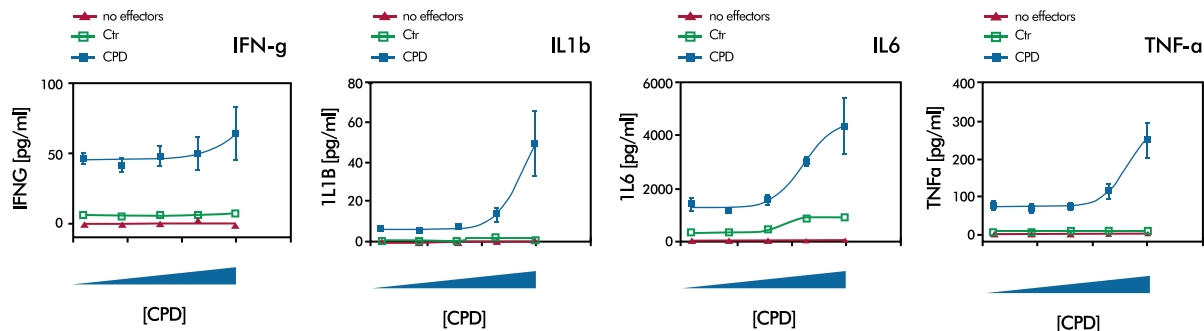
Representative data showing killing of luciferase-expressing target cells by CD14+ monocytes stimulated with increasing compound concentrations

# Multiplex Cytokine Assay

Measure cytokines released upon differentiation or activation of immune response

## Experimental workflow

- Isolation of CD14+ monocytes from PBMCs
- Co-culturing with target cells that express luciferase, at 10:1 (E:T ratio) effector to target ratio, meaning ten monocytes for every target cell
- Supernatant collection upon activation of immune response
- Cytokine level detection using electrochemiluminescent multiplex immunoassay (MSD)



*Representative data demonstrating cytokine release detected in cell culture supernatants from monocytes co-cultured with target cells with increasing compound concentrations response. The cytokine release is detected using MSD multiplex assay*



# Let's Discover Together

## Recombinant Proteins

- Kinase proteins
- Epigenetic proteins
- Substrates
- Custom-tailored protein production



## Target-Specific Assays

- Biochemical and cell-based assays
- Enzymatic activity testing
- Protein: Protein Interaction assays
- Receptor Biology



## Cellular Oncology

- 2D and 3D proliferation assays
- Drug combination screening
- Invasion and migration assays
- Angiogenesis assay



## Biophysical Assays

- Surface Plasmon Resonance
- Thermal Shift Assay
- Isothermal Titration Calorimetry
- Microscale Thermophoresis



## In Vivo Pharmacology

- In Vivo Hollow Fiber Model
- Xenograft models
- Orthotopic models
- Metastasis models



## Safety & Adme-Tox

- Cardiac Safety Panel
- CYP inhibition
- PK/PD studies
- In Vitro Safety Panel



## Integrated Drug Discovery

- Target research
- Hit identification
- Hit-to-Lead
- Lead optimization



## Biomarker Discovery

- Genomic biomarkers
- Protein biomarkers
- Immunophenotyping



## Immuno-Oncology

- In Vitro Killing Assays
- Syngeneic Mouse Models
- Proprietary Tumor Models
- Immunophenotyping



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Unveil the impact of your  
immunotherapy on macrophages  
in the tumor environment  
– **Let's discover together**

