DATA EXAMPLES

Differentiation of CD14$^+$ monocytes into M1 macrophages is confirmed by CD marker staining (CD14$^+$ CD80$^+$ CD163$^-$ CD206$^+$) and secretion of IL-12 and IL-10 (IL-12^{high} IL-10^{low}).

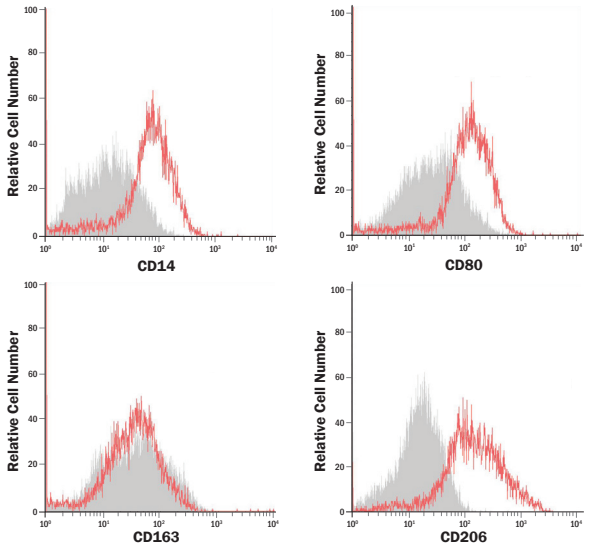

Figure 1: Phenotypic Analysis of human M1 Macrophages. Flow cytometry data show cell surface marker expression of human peripheral blood CD14$^+$ monocytes following differentiation using reagents included in the Human M1 Macrophage Differentiation Kit. On day 6 of the differentiation, cells were harvested and stained with antibodies for CD14, CD80, CD163, and CD206 (open histograms). Cell staining was gated using isotype control antibodies (filled histograms). M1 macrophages display a CD14$^+$ CD80$^+$ CD163$^-$ CD206$^+$ phenotype. All R&D Systems antibodies and corresponding catalog numbers used in this figure are shown in the table below.

SUGGESTED REAGENTS FOR FLOW CYTOMETRY

CATALOG #	DESCRIPTION
FAB3832F	Human CD14 Fluorescein-conjugated Antibody (Clone 134620), Mouse IgG_1
IC002F	Mouse IgG_1 Fluorescein Isotype Control (Clone 11711), Mouse IgG_1
FAB140P	Human B7-1/CD80 PE-conjugated Antibody (Clone 37711), Mouse IgG_1
IC002P	Mouse IgG_1 PE-conjugated Isotype Control (Clone 11711), Mouse IgG_1
FAB1607C	Human CD163 PerCP-conjugated Antibody (Clone 215927), Mouse IgG_1
IC002C	Mouse IgG_1 PerCP Isotype Control (Clone 11711), Mouse IgG_1
FAB25342A	Human MMR/CD206 APC-conjugated Antibody (Clone 685641), Mouse IgG_{2A}
IC003A	Mouse IgG_{2A} APC-conjugated Isotype Control (Clone 20102), Mouse IgG_{2A}

DATA EXAMPLES *CONTINUED*

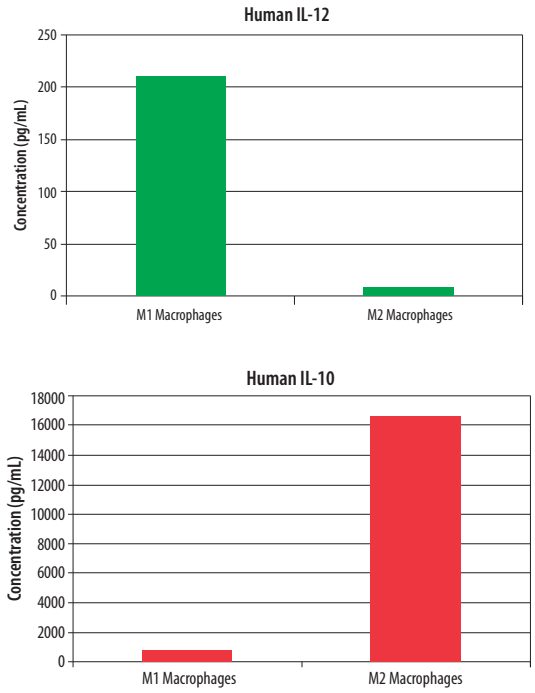

Figure 2: Differentiated Human M1 Macrophages Secrete IL-12. Human peripheral blood CD14$^+$ monocytes were differentiated for 6 days under M1 or M2 macrophage polarization conditions using reagents included in the CellXVivo™ Human M1 Differentiation Kit or the CellXVivo™ M2 Macrophage Differentiation Kit (R&D Systems, Catalog # CDK013). On day 6, M1 and M2 macrophages were stimulated with 1 µg/mL LPS for 24 hours. Cell culture supernatant was collected and cytokine secretion was determined using the Human IL-12 p70 Quantikine® HS ELISA Kit and the Human IL-10 Quantikine® ELISA Kit. All relevant R&D Systems Quantikine® ELISA kits and corresponding catalog numbers are listed below.

SUGGESTED REAGENTS FOR ELISA

CATALOG #	DESCRIPTION
HS120	Human IL-12 p70 Quantikine® HS ELISA Kit
D1000B	Human IL-10 Quantikine® ELISA Kit

CellXVivo™

Human M1 Macrophage Differentiation Kit

Catalog Number: CDK012

BACKGROUND

Macrophages derived from circulating inflammatory or resident monocytes are recruited to areas of tissue inflammation in response to injury or pathogenic insult. Monocyte-derived macrophages also replenish apoptotic resident macrophages to maintain tissue homeostasis (1). Macrophages can be classified into two major subtypes: type 1 or classically activated macrophages (M1) and type 2 or alternatively activated macrophages (M2) (2). *In vitro*, monocytes can be differentiated into M1 and M2 macrophages using GM-CSF or M-CSF, respectively. Each subtype can be characterized by their expression of a distinct set of cell-surface proteins and pro- or anti-inflammatory cytokines (3). M1 macrophages produce pro-inflammatory cytokines that combat pathogenic infection and reduce the infectivity of microbes. Prolonged or excessive activation of M1 macrophages can result in secondary damage to host tissue. M2 macrophages produce growth factors and anti-inflammatory cytokines to suppress the host immune response, promote wound healing and tissue remodeling, and improve metabolic and endocrine signaling within tissues (4, 5). The Human M1 Macrophage Differentiation Kit contains optimized components to differentiate human CD14$^+$ monocytes into CD14$^+$CD80$^+$CD163$^-$CD206$^+$ M1 macrophages. When activated using Lipopolysaccharide (LPS), these M1 macrophages have high expression of IL-12 and low IL-10 expression.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at ≤ -20 °C in a manual defrost freezer.
Do not use past kit expiration date.

COMPONENTS	PART #	# VIALS	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Serum-Free Base Media	390536	1 vial	May be stored at 2-8 °C under sterile conditions for up to 30 days or at -20 °C to -70 °C in a manual defrost freezer for up to 3 months.*
Recombinant Human GM-CSF	967990	1 vial	
Reconstitution Buffer 2	967553	1 vial	May be stored under sterile conditions for up to 3 months at 2-8 °C.*

* Provided this is within the expiration date of the kit.

OTHER MATERIALS & SUPPLIES REQUIRED

- MagCellect™ Human CD14$^+$ Cell Isolation Kit (R&D Systems®, Catalog # MAGH105, or equivalent).
- Ficoll-Hypaque™
- Tissue culture plates and/or flasks
- Penicillin (optional)
- Streptomycin (optional)
- Cell Dissociation Solution Non-enzymatic 1X (Sigma)
- Lipopolysaccharide (LPS) (Sigma, Catalog # L3024)
- Inverted microscope
- Hemocytometer
- 37 °C, 5% CO_2 incubator
- Centrifuge
- Pipettes and pipette tips

REFERENCES

1. Chávez-Galán, L. *et al.* (2015) Front. Immunol. **6**:263.
2. Mills, C.D. (2015) Front. Immunol. **6**:212.
3. Rey-Giraud, F. *et al.* (2012) PLoS One. **7**:e42656.
4. Wang, N. *et al.* (2014) Front. Immunol. **5**:614.
5. Rőszer, T (2015) Mediators Inflamm. **2015**:816460.

REAGENT PREPARATION

Serum-Free Base Media - Thaw at 2-8 °C or at room temperature.

Recombinant Human GM-CSF (500X) - Add 200 µL of Reconstitution Buffer 2 to the vial of Recombinant Human GM-CSF to produce Recombinant Human GM-CSF (500X).

Human M1 Macrophage Differentiation Media - Add 20 µL Recombinant Human GM-CSF (500X) to 10 mL of Serum-Free Base Media (**optional:** add Penicillin at 100 units/mL and Streptomycin at 100 µg/mL).

PROTOCOL FOR M1 MACROPHAGE DIFFERENTIATION

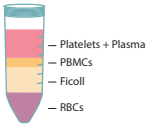
1. Isolate human peripheral blood mononuclear cells (PBMCs) from human blood using Ficoll-Hypaque density gradient centrifugation.
2. Enrich human CD14$^+$ monocytes from human PBMC using the MagCellect™ Human CD14$^+$ Cell Isolation Kit.
3. Suspend human CD14$^+$ monocytes at 2 x 10^6 cells/mL in Human M1 Macrophage Differentiation Media.
4. Add cells to plate.

SIZE	SUGGESTED CULTURE VOLUME
6-well plate	4 mL/well
24-well plate	1 mL/well
96-well plate	200 µL/well

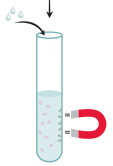
5. Incubate cells in a 37 °C, 5% CO_2 humidified incubator for 3 days.
6. On day 3, refresh the Human M1 Macrophage Differentiation Media. For each well of a 96-well plate, remove 100 µL of the media, for each well of a 24-well plate remove 500 µl of media, for each well of a 6-well plate remove 2 mL of media. For each plate size, replenish removed media with an equal volume of fresh Human M1 Macrophage Differentiation Media.
7. Incubate the cells as in step 5 for an additional 3 days.
8. On day 6 of differentiation, the differentiated M1 macrophages are ready for downstream applications.
9. Activation **(optional)**: Harvest cells using an enzyme free cell dissociation solution. Resuspend cells in Serum-Free Base Media and stimulate cells with 1 µg/mL LPS for 24 hours.
10. **Optional:** To verify M1 macrophage differentiation via flow cytometry, collect the cells using enzyme free cell disassociation solution. Process, stain, and analyze M1 macrophage marker expressions via flow cytometry, as shown in the Data Examples.
11. **Optional:** To verify M1 macrophage differentiation via ELISA, harvest cells using enzyme free cell disassociation solution. Resuspend cells in Serum-Free Base Media and stimulate cells with 1 µg/mL LPS for 24 hours. Analyze IL-12 and IL-10 in cell culture supernatant using the Human IL-12 p70 Quantikine® HS ELISA Kit (R&D Systems, Catalog # HS120) or the Human IL-10 Quantikine® ELISA Kit (R&D Systems®, Catalog # D1000B), respectively.

PROTOCOL OUTLINE

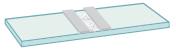
Isolate PBMCs from human blood.



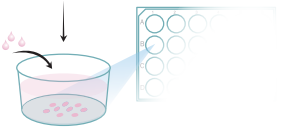
Enrich human CD14$^+$ monocytes from PBMCs (e.g., using magnetic cell selection).



Perform a cell count.



Suspend 2 x 10^6 cells/mL in Human M1 Macrophage Differentiation Media.
Culture the cells on plates for 6 days.
Add fresh Human M1 Macrophage Differentiation Media on day 3.



Verify M1 macrophage differentiation on day 6 by analyzing cell surface marker expression via flow cytometry.
M1 macrophages are ready for downstream application.