DATA EXAMPLES

Representative results obtained from the culture of CD14$^+$ enriched PBMCs with human monocyte-derived dendritic cell serum-free differentiation media over a 7-day or a 10-day (with maturation) period are shown in the following figures.

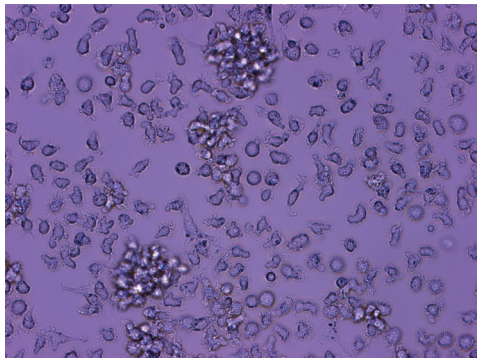

Figure 1: Morphology of Immature Human Dendritic Cells Cultured in Differentiation Media for 7 days.

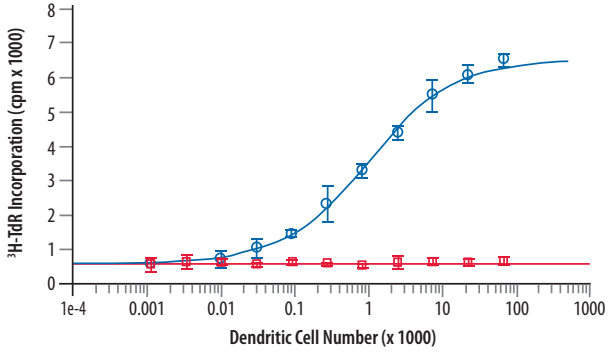

Figure 2: Mature Dendritic Cells Induce Proliferation of Allogeneic T-cells. Serial dilutions of day 10 TNF-α treated mature dendritic cells were incubated with allogeneic (blue) or autologous (red) human T cells for 3 days. ^{3}H-Thymidine (^{3}H-TdR) was added to the culture for the final 18 hours. Cells were harvested and the incorporation of ^{3}H-TdR was measured using a scintillation counter. Results are presented as the mean cpm of triplicates.

REFERENCES

1. Soloff A.C. and S.M. Carrett-Boyes (2010) Cell Res. **20**:872.
2. Harman, A.N. *et al.* (2013) J. Immunol. **190**:66.
3. Tailor, P. *et al.* (2006) Cell Res. **16**:134.
4. Pulendran, B. *et al.* (2010) Nat. Immunol. **8**:647.

DATA EXAMPLES *CONTINUED*

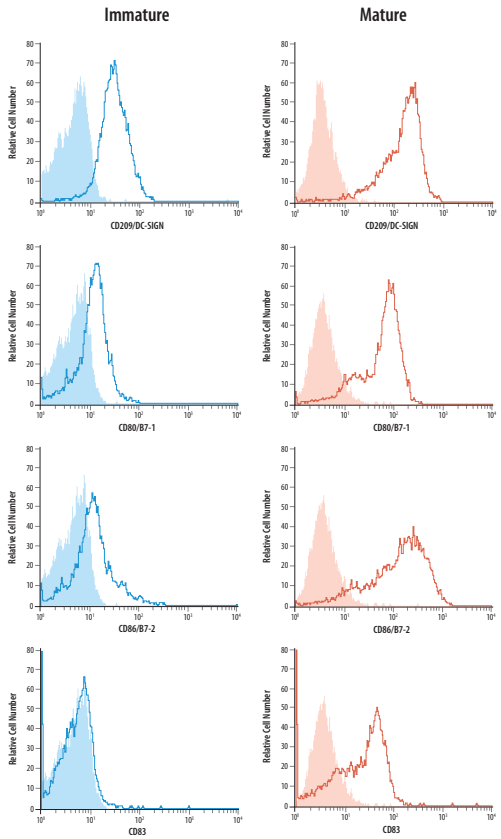

Figure 3: Phenotypic Analysis of Cultured Immature and Mature Monocyte-derived Dendritic Cells. Following culture in complete monocyte-derived differentiation media provided in this kit, day 7 immature dendritic cells (left) and day 10 TNF-α treated mature dendritic cells (right) were stained with the indicated antibodies for DC-SIGN, CD80, CD86, CD83 (open histograms), or an appropriate isotype control antibody (filled histograms). Stained cells were analyzed using a Becton Dickinson FACSCalibur™. All R&D Systems antibodies and corresponding catalog numbers used in this figure are shown below.

CATALOG #	DESCRIPTION
MAB161	Human DC-SIGN/CD209 MAb (Clone 120507), Mouse IgG_{2B}
MAB140	Human B7-1/CD80 MAb (Clone 37711), Mouse IgG_1
MAB141	Human B7-2/CD86 MAb (Clone 37301), Mouse IgG_1
MAB1774	Human CD83 MAb (Clone HB15e), Mouse IgG_1
MAB004	Mouse IgG_{2B} Isotype Control (Clone 20116)
MAB002	Mouse IgG_1 Isotype Control (Clone 11711)
F0102B	Goat F(ab)2 Anti-Mouse IgG (H+L) Phycoerythrin

CellXVivo™

Human Monocyte-derived Dendritic Cell Differentiation Kit

Catalog Number: CDK004

BACKGROUND

Dendritic cells (DCs) are key mediators of both innate and adaptive immune responses. Immature DCs express specific pattern recognition receptors that serve as expression markers and allow for the capture and processing of foreign antigens following infection (1, 2). Upon activation, immature dendritic cells mature and increase the expression of class II MHC and co-stimulatory molecules important for effective antigen presentation to naïve T cells (3). Cytokines produced by DCs can also promote the differentiation of CD4$^+$ T helper cells as part of immune activation (4). The Human Monocyte-derived Dendritic Cell Differentiation Kit contains the media and cytokine components to generate immature and mature dendritic cells from CD14$^+$ peripheral blood mononuclear cells (PBMCs) under serum free conditions.

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 Dendritic Cell Base Media	390536	1 vial (100 mL)	Store 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 IL-4	967569	1 vial (3.5 µg)	
Recombinant human GM-CSF	967570	1 vial (5 µg)	
Recombinant human TNF-α	967571	1 vial (2 µg)	
Reconstitution Buffer 2	967553	2 vials	Store at 2-8 °C under sterile conditions for up to 3 months.*

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

OTHER MATERIALS & SUPPLIES REQUIRED

- Ficoll-Hypaque™
- MagCellect™ Human CD14⁺ Cell Isolation Kit (R&D Systems, Catalog# MAGH105, or equivalent)
- Penicillin/Streptomycin (optional)
- Tissue culture flasks and/or plates
- Pipettes and pipette tips
- Inverted microscope
- Hemocytometer
- 37 °C, 5% CO_2 incubator
- Centrifuge

REAGENT PREPARATION

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

Recombinant human IL-4 (200X) - Add 500 µL of Reconstitution Buffer 2 to Recombinant human IL-4 to produce Recombinant human IL-4 (200X).

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

Recombinant human TNF-α (200X) - Add 250 µL of Reconstitution Buffer 2 to Recombinant human TNF-α to produce Recombinant human TNF-α (200X).

Differentiation Media - Add Recombinant human IL-4 (200X) and Recombinant human GM-CSF (200X) to a final concentration of 1X to the desired amount of Serum-Free Dendritic Cell Base Media. (*e.g.,* for every 10 mL of base media, add 50 µL each of Recombinant human IL-4 (200X) and Recombinant human GM-CSF (200X).

Note: *TNF-α can be added to this media during the maturation steps. This media does not contain antibiotics, but they can be added when desired.*

PROTOCOL FOR DENDRITIC CELL DIFFERENTIATION

1. Isolate human peripheral blood mononuclear cells (PBMCs) from human blood using Ficoll-Hypaque density gradient centrifugation.
2. Isolate CD14⁺ cells from human PBMCs using the MagCellect Human CD14⁺ Cell Isolation Kit.
3. Resuspend human CD14⁺ cells at 1 x 10^6 cells/mL in Differentiation Media. Add the cell suspension to the tissue culture flask or tissue culture plate as suggested below.

Size	Suggested Culture Volume
75 cm^2 tissue culture flask	20 mL
25 cm^2 tissue culture flask	10 mL
6-well tissue culture plate	3 mL/well
24-well tissue culture plate	1 mL/well

4. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator for 3 days.
5. On day 3, change the media by removing half of the media from the well or flask and replenishing with the same volume of fresh Differentiation Media.

   Note: *Use caution when removing the media to avoid aspirating cells. Alternatively, the spent media can be transferred to a centrifuge tube and then centrifuged at 200 x g for 5 minutes. Any cell pellet formed can be resuspended in fresh media and added back to the same well or flask.*
6. Incubate the cells as in step 4 for an additional 2 days.
7. On day 5, repeat step 5 to change the media.
8. Incubate the cells as in step 4 for an additional 2 days.
9. On day 7, immature dendritic cells can be observed and are ready to be used in the desired application. If maturation is desired, continue to Step 10.
10. On day 7, repeat step 5 to change the media. Dendritic cell maturation can be induced with your preferred maturation agents. In this protocol, maturation is induced by adding Recombinant human TNF-α (200X) to a final concentration of 1X to the cell suspension. (*e.g.,* for every 10 mL of Differentiation Media, add 50 µL of Recombinant human TNF-α (200X).
11. Incubate the cells as in step 4 for an additional 3 days.
12. On day 10, mature dendritic cells can be observed and are ready to be used in the desired application.

PROTOCOL OUTLINE