DATA EXAMPLES

The following figures show representative results obtained from bone marrow cells cultured in Differentiation Media from the CellXVivo™ Mouse Dendritic Cell Differentiation Kit over 5 days to produce immature dendritic cells, or over 6 days to produce mature dendritic cells.

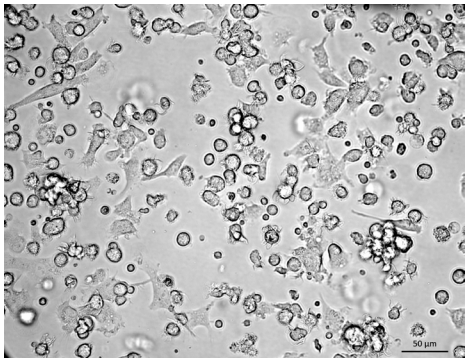

Figure 1: Morphology of Immature Mouse Dendritic Cells Cultured in Differentiation Media for 5 days.

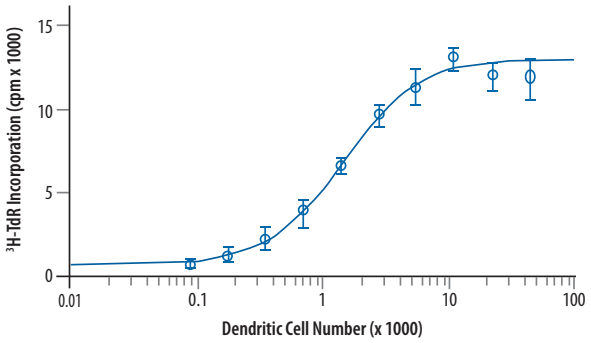

Figure 2: Kit-derived Mature Mouse Dendritic Cells Induce Proliferation of Allogeneic T Cells. Serial dilutions of TNF-α-treated mature dendritic cells were incubated with allogeneic mouse T cells for 3 days. ^{3}H-Thymidine (^{3}H-TdR) was added for the final 18 hours of the culture. Cells were harvested 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*

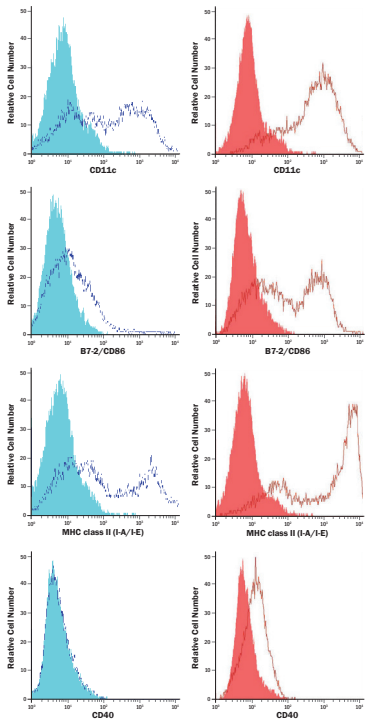

Figure 3: Phenotypic Analysis of Cultured Immature and Mature Mouse Dendritic Cells. Immature dendritic cells (left, blue) and TNF-α-treated mature dendritic cells (right, red), derived from bone marrow cells cultured with the CellXVivo™ Mouse Dendritic Cell Differentiation Kit, were stained for flow cytometry with antibodies for CD11c, B7-2/CD86, MHC class II (I-A/I-E), or CD40 (open histograms). An appropriate isotype antibody (filled histograms) was used as a control. Immature dendritic cells have the phenotype of CD11c$^+$, with intermediate expression of B7-2/CD86, MHC class II, and little to no CD40. Mature dendritic cells have the phenotype of CD11c$^+$, with increased expression of B7-2/CD86, MHC class II, and CD40. All R&D Systems antibodies and corresponding catalog numbers used in this figure are highlighted below.

SUGGESTED REAGENTS FOR FLOW CYTOMETRY

DESCRIPTION	FL	PE	PERCP	APC
Mouse CD11c MAb (Clone N418), Hamster IgG	FAB9501G-25 FAB9501G-100	FAB9501P-25 FAB9501P-100	FAB9501C-25 FAB9501C-100	FAB9501A-25 FAB9501A-100
MHC class II (I-A/I-E, Clone M5/114.15.2), Rat IgG_{2B}	FAB118F			FAB118A
Mouse B7-2/CD86 MAb (Clone GL1), Rat IgG_{2A}	FAB741G	FAB741P	FAB741C	FAB741A
Mouse CD40 MAb (Clone 1C10), Rat IgG_{2A}	FAB440F			

CellXVivo™

Mouse Dendritic Cell Differentiation Kit

Catalog Number: CDK008

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 CellXVivo™ Mouse Dendritic Cell Differentiation Kit contains the media and cytokine components to generate immature and mature dendritic cells from mouse bone marrow.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:
USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:
UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

www.RnDSystems.com

R&D SYSTEMS a bio-techne brand

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at -20 °C to -70 °C in a manual defrost freezer. Do not use past kit expiration date.

COMPONENTS	PART #	# VIALS	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse Dendritic Cell Base Media	967852	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 Mouse GM-CSF	967853	1 vial	
Recombinant Mouse IL-4	967854	1 vial	
Recombinant Mouse TNF-α	967855	1 vial	
10X Erythrocyte Lysing Buffer	895127	1 vial (1 mL)	Store at 2-8 °C under sterile conditions for up to 3 months.*
Reconstitution Buffer 1	967552	2 vials (1 mL/each)	

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

OTHER MATERIALS & SUPPLIES REQUIRED

- Laboratory mice
- Syringe and needle
- Deionized or distilled water
- PBS
- Tissue culture flasks and/or plates
- Inverted microscope
- Hemocytometer
- 37 °C, 5% CO_2 incubator
- Centrifuge
- Pipettes and pipette tips

REAGENT PREPARATION

Mouse Dendritic Cell Base Media - Thaw at room temperature.

Reconstitution Buffer 1 - Thaw at room temperature.

Recombinant Mouse GM-CSF (200X) - Add 500 µL of Reconstitution Buffer 1 to Recombinant Mouse GM-CSF to produce Recombinant Mouse GM-CSF (200X).

Recombinant Mouse IL-4 (200X) - Add 500 µL of Reconstitution Buffer 1 to Recombinant Mouse IL-4 to produce Recombinant Mouse IL-4 (200X).

Recombinant Mouse TNF-α (200X) - Add 250 µL of Reconstitution Buffer 1 to Recombinant Mouse TNF-α to produce Recombinant Mouse TNF-α (200X).

Erythrocyte Lysing Buffer - Thaw 10X Erythrocyte Lysing Buffer at room temperature. Add 1 mL of 10X Erythrocyte Lysing Buffer to 9 mL deionized or distilled water to produce 10 mL of Erythrocyte Lysing Buffer.

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

Note: *Recombinant Mouse TNF-α can be added to this media during the maturation steps.*

PROTOCOL FOR DENDRITIC CELL DIFFERENTIATION

1. Prepare a single cell suspension of mouse bone marrow cells.
2. Remove red blood cells by resuspending bone marrow cells in 2.5 mL of Erythrocyte Lysing Buffer and incubate at room temperature for approximately 5 minutes. Stop the reaction by adding 10 mL PBS and centrifuge at 200 x g for 5 minutes.
3. Discard supernatant and resuspend mouse bone marrow 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
T-75 cm^2 tissue culture flask	20 mL/flask
T-25 cm^2 tissue culture flask	7.5 mL/flask
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 3/4 of the media from the flask or plate 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 flask or wells.*
6. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator for an additional 2 days.
7. On day 5, immature dendritic cells are ready. Collect the non-adherent and loosely adherent cells for use in your desired downstream application. If maturation is desired, continue to Step 8.
8. On day 5, resuspend immature dendritic cells in fresh Differentiation Media at 1 x 10^6 cells/mL and re-plate in a new tissue culture flask or tissue culture plate. In this protocol, dendritic cell maturation is induced by adding TNF-α (200X) to a final concentration of 1X to the cell suspension (*e.g.*, for every 1 mL Differentiation Media, add 5 µL of Recombinant Mouse TNF-α (200X)).

   Note: *Alternatively, dendritic cell maturation can be induced with your preferred maturation agents.*
9. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator for an additional 1 day.
10. On day 6, mature dendritic cells can be observed. Collect suspended and loosely adherent cells which are ready to be used in the desired application.

PROTOCOL OUTLINE