StemXVivo® Serum-Free Dendritic Cell Base Media

Serum-Free Base Media for Human Dendritic Cell Culture

Catalog Number: CCM003

Volume: 250 mL

PRODUCT DESCRIPTION

The StemXVivo Serum-Free Dendritic Cell Base Media is formulated and optimized for the culture and differentiation of human dendritic cells. This product is supplemented with sodium bicarbonate but does not contain antibiotics.

INTENDED USE

The StemXVivo Serum-Free Dendritic Cell Base Media must be used with cytokine/growth factor supplements for the desired cell culture application. The cytokine/growth factor combinations used depends upon the experimental design of each researcher.

STABILITY & STORAGE

Upon receipt, this media should be stored at ≤ -20 °C in a manual defrost freezer. The media can be thawed at 2-8 °C or at room temperature. Thawed media can be aliquoted and stored at ≤ -20 °C in a manual defrost freezer for up to 3 months or used within a month when stored in the dark at 2-8 °C. Avoid repeated freeze-thaw cycles.

PRECAUTION

The human origin-derived components used in this product have been derived from human plasma, which has been tested and found negative for antibodies to HIV-1/2, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV). However, the medium should be handled as if potentially infectious. Safe laboratory procedures should be followed and protective clothing should be worn when handling this media. The acute and chronic effects of over-exposure to this media are unknown.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- This reagent should not be used beyond the expiration date indicated on the label.
- Results may vary due to variations between dendritic cell precursors/progenitor cells derived from different donors.

OTHER MATERIALS REQUIRED

- Human $CD14^+$ monocytes isolated from peripheral blood mononuclear cells using the MagCellect™ Human $CD14^+$ Cell Isolation Kit (R&D Systems, Catalog # MAGH105)
- Cytokines: Recombinant Human GM-CSF (R&D Systems, Catalog # 215-GM) and Recombinant Human IL-4 (R&D Systems, Catalog # 204-IL)
- LPS
- Gentamicin (Catalog # B20192)
- Tissue culture flasks or plates
  - 25 cm^2 and 75 cm^2 tissue culture flasks
  - 6-well and 24-well tissue culture plates
- 15 mL centrifuge tubes
- Serological pipettes
- Pipettes and pipette tips
- Hemocytometer
- Centrifuge
- Vortex mixer
- Inverted Microscope
- 37 °C and 5% CO_2 incubator

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PROCEDURE FOR THE *EX VIVO* GENERATION OF HUMAN MONOCYTE DERIVED DENDRITIC CELL FROM CD14$^+$ MONOCYTES

The protocol below outlines differentiation of mature, monocyte-derived dendritic cells (MoDC) from highly purified CD14$^+$ monocytes over a 9 day period. Differentiation of the CD14$^+$ monocytes into immature MoDC can be observed after 5-7 days of culture in StemXVivo® Serum-Free Dendritic Cell (DC) Base Media with Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) and Interleukin 4 (IL-4). A maturation agent, such as lipopolysaccharides (LPS) is added on day 7 for 48 hours to induce DC maturation. The maturation stage and purity of DCs in each experiment may vary due to variation in the primary cells obtained and the purity of the starting cell population. Individual researchers may modify the protocol depending upon their experimental design. This StemXVivo Serum-Free DC Base Media is also applicable to the preparation of human DCs from other sources.

Note: *This protocol must be read in its entirety before using this product.*

PROCEDURE

1. Thaw the needed volume of the StemXVivo Serum-Free Dendritic Cell Base Media at 2-8 °C or room temperature. Thoroughly mix the thawed media by vortexing and warm the media in a 37 °C incubator.

   Note: *When handling biohazard materials such as human blood, safe laboratory procedures should be followed and protective clothing should be worn.*

2. Prepare the CD14$^+$ cells from peripheral blood mononuclear cells according to the instructions in the MagCellect™ CD14$^+$ Cell Isolation Kit.
3. Resuspend the purified CD14$^+$ cells in StemXVivo Serum-Free Dendritic Cell Base Media, count the cells and adjust the cell density to 1 x 10^6 cells/mL.
4. Supplement the media with 50 µg/mL of Gentamicin, 50 ng/mL of recombinant human GM-CSF and 35 ng/mL of recombinant human IL-4.
5. Add the cell suspension to the tissue culture flask or tissue culture plate as suggested.

Size	Suggested Culture Volume
75 cm^2 TC flask	30 mL
25 cm^2 TC flask	10 mL
24-well TC plate	1 mL per well
6-well TC plate	3 mL per well

   Note: *It is recommended to put the plate(s) in a humidified container to avoid evaporation of the media.*

6. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator for 7 days. Change the media every three days by removing half of the media from the plate and replenishing with the same volume of fresh media supplemented with Gentamicin and cytokines.

   Note: *Use caution when removing the media to avoid aspirating cells. Alternatively, the spent media can be transferred to a sterile 15 mL centrifuge tube and then centrifuged at 300 x g for 8 minutes. Any cell pellet formed at the bottom of the 15 mL centrifuge tube can be resuspended in fresh media supplemented with Gentamicin and cytokines and added back to the same well or flask.*

7. Immature MoDC can be observed from day 5 to day 7. If maturation is desired, it can be induced with maturation agents such as LPS, TNF-α, and CD40L. In this protocol, maturation of MoDC is induced by adding 1 µg/mL LPS to the cell suspension on day 7 and incubating for 48 hours.
8. Characteristics of the immature and mature DCs can be determined by a number of assays including flow cytometric analysis of the surface markers expressed on DCs (Table 1), the cytokine production profile of activated DCs, FITC-Dextran uptake to assay phagocytosis, or the mixed leukocyte reaction (MLR).

Cell Surface Marker	Immature MoDC	Mature MoDC
B7-1/CD80	+	++
B7-2/CD86	++	++++
CD83	+	+++
MHC class I	+	++
MHC class II	+++	++++

DATA EXAMPLES

Representative results obtained from the culturing of MoDC enriched from peripheral mononuclear cells with the StemXVivo® Serum-Free Dendritic Cell Base Media (R&D Systems®, Catalog # CCM003) in the presence of GM-CSF and IL-4 over a 7-day or a 9-day (with maturation) period are shown in the following figures.

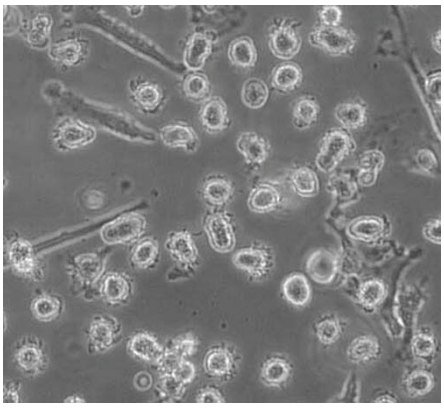

Figure 1: Differentiation of CD14+ Monocytes into Dendritic Cells. LPS-matured monocyte-derived dendritic cells were obtained after CD14$^+$-enriched monocytes were cultured for 9 days in StemXVivo Serum-Free Dendritic Cell Base Media (R&D Systems, Catalog # CCM003) supplemented with Recombinant Human GM-CSF (R&D Systems, Catalog # 215-GM) and Recombinant Human IL-4 (R&D Systems, Catalog # 204-IL).

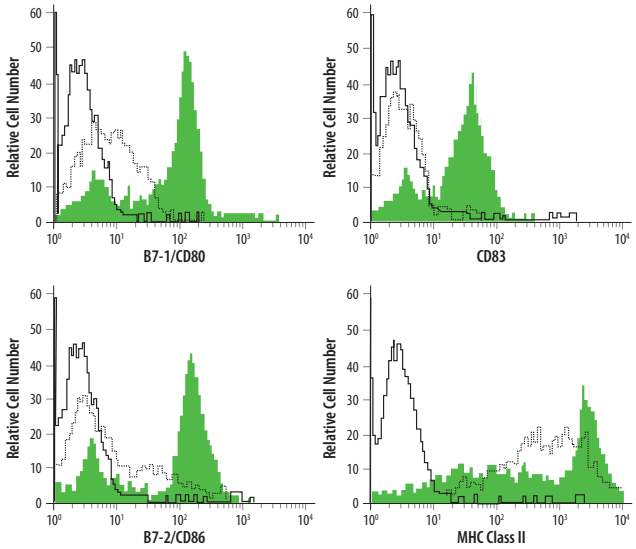

Figure 2: Phenotypic Analysis of Cultured Monocyte-derived Dendritic Cells Before and After LPS-induced Maturation. Immature monocyte-derived dendritic cells (MoDCs) (open histograms; dotted line) were obtained after CD14$^+$-enriched monocytes were cultured for 7 days in StemXVivo Serum-Free Dendritic Cell Base Media supplemented with Recombinant Human GM-CSF and Recombinant Human IL-4. Mature monocyte-derived dendritic cells (green filled histograms) were cultured under the same conditions for 7 days and then induced with LPS for an additional 48 hours. Day 7 immature MoDCs and day 9 LPS-treated MoDCs were stained with a FITC-conjugated Mouse Anti-Human B7-1/CD80 Monoclonal Antibody (R&D Systems, Catalog # FAB140F), a FITC-conjugated Mouse Anti-Human CD83 Monoclonal Antibody (R&D Systems, Catalog # FAB1774F), a FITC-conjugated Mouse Anti-Human B7-2/CD86 Monoclonal Antibody (R&D Systems, Catalog # FAB141F), an Anti-MHC Class II Antibody, or an appropriate isotype control antibody (empty histogram with solid line).

DATA EXAMPLES *CONTINUED*

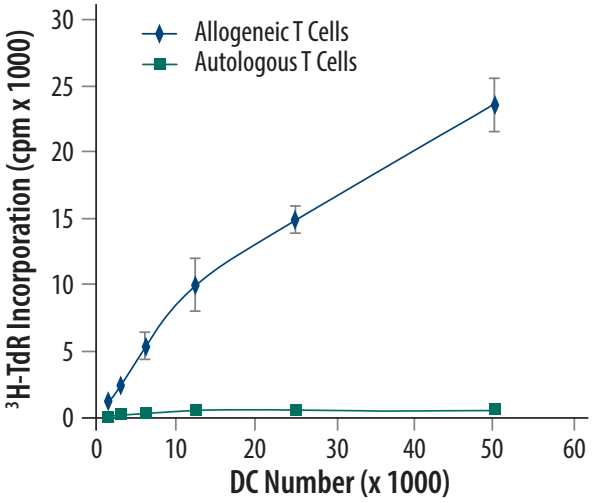

Figure 3: Mature Monocyte-derived Dendritic Cells Induce Proliferation of Allogeneic T Cells. $CD14^+$ monocytes were cultured for seven days in StemXVivo® Serum-free Dendritic Cell Base Media supplemented with Recombinant Human GM-CSF and Recombinant Human IL-4. The cells were subsequently treated with LPS for an additional 48 hours to induce dendritic cell maturation. Graded doses of mature monocyte-derived dendritic cells were incubated with 1 x 10^5 autologous or allogeneic $CD3^+$ T cells for 5 days. ^{3}H-thymidine (^{3}H-TdR) was added to the culture for the final 18 hours and T cell proliferation was measured using a scintillation counter. Results are presented as the mean cpm obtained from three experiments.