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Dendritic Cell (DC) and T Cell Assay from Matched PBMCs

Instructions for use

Safety statements

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

WARNING: LONZA PRIMARY CELLS CONTAIN HUMAN SOURCE MATERIAL; TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-I, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV-1, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure to potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th edition. If you require further information, please contact your site safety officer or Scientific Support.

Preparation of reagents

All work should be done in a laminar flow hood. Decontaminate the external surfaces of all supplement vials and the medium bottles with ethanol or isopropanol.

1. **Release buffer**
   Prepare a stock bottle of 5 mM EDTA in PBS without Ca^{2+} or Mg^{2+} by aseptically adding 10 mL of 0.5 mM EDTA to 990 mL PBS. This buffer can be stored at 2-8ºC for 1 year.
2. **Adherence Medium**
   Supplement X-VIVO® 15 Serum-free Hematopoietic Cell Medium with 0.5% heat inactivated human plasma containing 50 U/mL Heparin. Keep media at 4°C.
3. **DC Culture Medium Base**
   Prepare culture medium base during incubation for plastic adherence. **Do not add cytokines to culture medium base more than 30 minutes prior to completion of plastic adherence incubation.** Supplement X-VIVO® 15 Serum-free Hematopoietic Cell Medium with 0.5% heat inactivated human plasma containing 50 U/mL Heparin. Add the following cytokines to the culture medium base such that the final concentration is as follows: IL-4 (1000 U/mL) and GM-CSF (800 U/mL). Only make enough culture base medium as needed. Once cytokines are added to medium do not store media for longer than 30 minutes. Media supplemented with cytokines should be made fresh each time prior to use.
4. **IL-2 Medium**
   Prepare stock IL-2 media by adding 1 µL of 5 µg/mL working solution IL-2 to 1 mL X-VIVO™ ® 15 for a final concentration of 5 ng/mL.
5. **CFSE Reagent**
   Follow company protocols for stock and working solutions of CFSE stain. Working solution should be at a final concentration of 5 µM.

Culturing and maturation of DCs

NOTE: All work is to be performed in a laminar flow hood.

1. Pipette 10 mL of pre-warmed X-VIVO® 15 Serum-free Hematopoietic Cell Medium into a 15 mL conical tube.
2. Thaw PBMCs in a water bath at 37ºC for ~2 min and pipette cells into the X-VIVO® 15 Serum-free Hematopoietic Cell Medium in the 15 ml conical from step 1.
3. Wash cell vial with 1 mL X-VIVO® 15 Serum-free Hematopoietic Cell Medium and add wash to 15 mL conical from step 2.
4. Centrifuge cells at 300xg for 10 minutes at 4ºC.
5. Resuspend pellet in 1 mL X-VIVO® 15 Serum-free Hematopoietic Cell Medium and count using Trypan Blue and a hemocytometer.
6. After count, plate PBMCs at $5x10^6$ cells/mL in adherence medium into appropriate flask. Adjust accordingly to flask size.
7. Place the planted flasks at 37ºC ± 2ºC, 5% CO_2 ± 2% CO_2 for 2 hours for adherence. Verify adherence of cells under the microscope. Cells should not be free floating or move with the media as the plate is inspected under the microscope.
8. Rinse the adherent cells with PBS (without Ca^{2+}, Mg^{2+}, or EDTA as it will cause cells to detach),

by gently adding PBS to flask without disturbing attached cells. Gently tap flask and swirl wash around the flask.

9. Repeat wash for a total of 2 washes per flask.
10. Feed adherent cells with culture medium base containing cytokines. For 24 well plates use 1 mL culture medium base containing cytokines. Adjust volume of culture medium base containing cytokine accordingly. Place the flasks at 37ºC ± 2ºC, 5% CO_2 ± 2% CO_2 for 3 days.
11. On day 3, collect media and nonadherent cells and spin at 300xg for 10 minutes.
12. Resuspend cell pellet in 1 mL culture medium base containing cytokines and reseed back into culture.
13. To mature and activate the DCs on day 3, add 100 µg/mL LPS to each culture. Place the flasks at 37ºC ± 2ºC, 5% CO_2 ± 2% CO_2 for 24 hours.

Note: Pre-isolated human dendritic cells (Cat: CC-2701) are available for purchase from Lonza. If using Lonza dendritic cells, start protocol from step 13. Use of pre-isolated Lonza dendritic cells results in a reduction of time of 3 days.

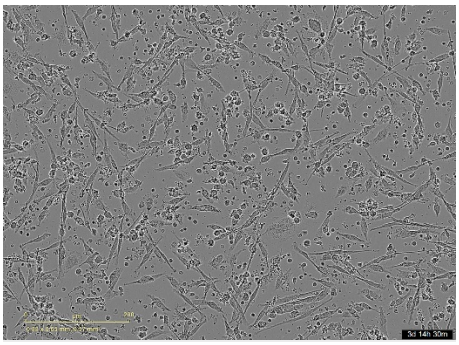

Figure 1: Mature DC morphology after 24 hour exposure to LPS. Mature DCs should have a rough surface with multiple pseudopodia.

14. Prior to harvest on day 4, ensure that PBS without Ca^{2+} or Mg^{2+} plus 5 mM EDTA is **ice cold**. Remove culture medium and any unattached cells from each flask.
15. Briefly rinse flasks with ice cold PBS without Ca^{2+} or Mg^{2+} plus 5 mM EDTA. Place rinse in conical with the media collected from step 14.
16. Add additional ice-cold PBS without Ca^{2+} or Mg^{2+} plus 5 mM EDTA to each flask and incubate for 10 minutes.
17. After incubation, tap sides of flask and wash flask to assist with detachment. Remove all detached cells and place them in a conical tube. Centrifuge cells at 300xg for 10 minutes.
18. Resuspend cells in 1 mL X-VIVO® 15 Serum-free Hematopoietic Cell Medium.

DC:T Cell Assay

NOTE: All work is to be performed in a laminar flow hood. After addition of CFSE, work should be protected from light.

1. Pipette 10 mL of pre-warmed X-VIVO® 15 Serum-free Hematopoietic Cell Medium into a 15 mL conical.
2. Thaw PBMCs at 37ºC for ~2 min and pipette cells into X-VIVO® 15 Serum-free Hematopoietic Cell Medium in the 15 mL conical from step 1.
3. Wash cell vial with 1 mL X-VIVO® 15 Serum-free Hematopoietic Cell Medium and add wash to conical.
4. Centrifuge cells at 300xg for 10 minutes at 4ºC.
5. Resuspend pellet in 1 mL PBS and count using Trypan Blue and a hemocytometer.
6. Purify $CD4^+$ T cells using Miltenyi CD4 Microbeads and following manufacturer's protocol.
7. Once purified, resuspend cells in 1 mL PBS and count using Trypan Blue and a hemocytometer.

Note: Pre-isolated human naïve CD4 T cells (Cat: 4W-202) are available for purchase from Lonza. If using Lonza naïve CD4 T cells, thaw the cells as described in steps 2-5 and proceed with protocol from step 8. Use of Lonza naïve CD4 T cells results in a reduction of reagents and time.

8. After count, remove appropriate number of cells and place into a new 50 mL conical for CFSE staining. For example, one reaction requires $1x10^6$ cells/mL. Adjust according to number of reactions and reaction volume.
9. Add 1 mL CFSE working solution (5 µM) per $1x10^6$ cells and mix by vortexing.
10. Incubate 20 minutes protected from light at RT.
11. After incubation, add X-VIVO® 15 Serum-free Hematopoietic Cell Medium at 4X the volume as CFSE stain to stop the reaction. Incubate for 5 minutes at room temperature.
12. Centrifuge cells 300xg for 10 minutes. Once T cells have been stained, allow the cells to rest at room temperature a minimum of 10 minutes prior to stimulation.
13. During incubation, prepare assay plate. For a 24-well plate the following volumes and controls are recommended:
    a. Negative control wells: 900 µL X-VIVO® 15 Serum-free Hematopoietic Cell Medium
    b. Positive control wells: 850 µL IL-2 Medium + 50 µL CD3/CD28 Dyna Beads (ThermoFisher 11131D)
    c. DC and T cell wells: Co-culture stained T cells with mature DCs at a

ratio 10:1 (T cell: DC) in 800 µL X-VIVO® 15 Serum-free Hematopoietic Cell Medium

d. DC and T cell and IL-2 wells: Co-culture stained T cells with mature DCs at a ratio 10:1 (T cell: DC) in 800 µL IL-2 Medium

14. Add T cells to each well (final concentration of $1x10^6$ cells/mL – adjust according to tissue plate size). For 24 well plates, add 100 µL cells.
15. Add DCs to each well (final concentration of $1x10^5$ cells/mL – adjust according to tissue plate size). For 24 well plates, add 100 µL cells.
16. Allow assay to incubate for 4 days at 37°C, 5% CO_2 to allow for T cell proliferation.
17. On day 4, collect T cell suspension from cultures and stain with Live/dead fixable cell dye for 20 minutes and analyze via FACS.
18. Assess cells via CFSE intensity (MFI of FITC channel) to determine T cell proliferation (i.e., decreased CFSE signal indicates high T cell proliferation)

Note: Assay can be performed with pre-isolated Lonza dendritic cells (Cat: CC-2701) and Lonza $CD4^+$ naïve T cells (Cat: 4W-202). Ideally allogeneic or autologous matched cells would be needed to perform the assay in this manner.

Ordering information

Catalog no.	Description	Size
CC-2702	Human Peripheral Blood Mononuclear Cells (hPBMC), Cryopreserved	≥50 million cells
CC-2701	NHDC- Human Dendritic Cells	≥2.5 million cells
4W-202	Human CD4+/CD45RA+ Naïve T cells, cryopreserved	≥5 million cells
04-418Q	X-VIVO™ ® 15 Serum -free Hematopoietic Cell Medium	1L, complete with L-Glutamine, gentamicin, and phenol red, xenofree
BE17-516Q	Phosphate Buffered Saline (1X) without Calcium and Magnesium	1 L

CD4 MicroBeads, human (Miltenyi Biotec 130-045-101) mentioned is a product from Miltenyi Biotec.

LPS (Sigma L5668-2ML) mentioned is a product from Millipore Sigma.

GM-CSF (PeproTech 300-03) mentioned is a product from PeproTech.

IL-4 (R&D Systems 204-IL-010) mentioned is a product from R&D Systems.

IL-2 (R&D Systems 202-IL-500) mentioned is a product from R&D Systems.

Dyna Beads (ThermoFisher 11131D) mentioned is a product of ThermoFisher Scientific.