

abcam

BMDC isolation

Abcam protocol

Bone marrow-derived dendritic cell isolation

This protocol will guide you through isolating immature bone marrow-derived dendritic cells (BMDCs) from mice.

Reagents and equipment

EDTA
Ethanol (70%)
Granulocyte macrophage colony-stimulating factor (GM-CSF) (ab9742)
Heat-inactivated fetal bovine serum (HI-FBS)*
L-glutamine
Penicillin
Phosphate buffered saline (PBS)
Roswell Park Memorial Institute medium (RPMI) medium
Streptomycin
β -Mercaptoethanol (β -ME) (tissue culture-grade)
Trypan blue
Sterile Kimwipes™
Sterile instruments (scissors and forceps)
Bacteriological petri dishes (100 mm x 15 mm)
Syringe (3 mL) and needle (31½ gauge)

*If FBS is not heat-inactivated, inactivate it by placing in a 56°C water bath for an hour and then cool to room temperature

Solutions

Prepare the following solution before beginning the experiment.

RPMI complete (R10)

Add the following reagents to RPMI medium before filter-sterilizing into a sterile, endotoxin-free bottle through a 0.22 μm filter.

HI-FBS	10%
L-glutamine	2 mM
Penicillin	100 U/mL
Streptomycin	100 $\mu\text{g}/\text{mL}$
β -ME	50 μM

Procedure

Isolation

- ! Ensure that all the reagents and samples are kept on ice from steps 1–6 as this has a positive impact on cell viability.
 - ! In order to maintain sterility, perform all steps in a laminar flow hood with sterile utensils. Watch our [video guide on aseptic technique](#).
1. Cut the back legs above the hip joint in order to access the femur and tibia, leaving knee and ankle joints in place, and debride the muscle and tissue from by rubbing with Kimwipes™.
 - ! The femur tends to have more marrow than the tibia.
 - ! Use only intact bones, as the bone marrow will not be sterile if they break.
 2. Fill a petri dish with 70% **ethanol** and dip the cleaned bones into it for 5–10 seconds to sterilize their exteriors before keeping them in a sterile tube on ice until all the bones are flushed.
 - ! The bones can be left in **ethanol** for longer while each bone is flushed, but be aware that it can soak into the bone and kill viable cells.
 3. In a laminar flow hood using sterile utensils, cut both ends of the bone with scissors as close to the joints as possible. Fill a syringe with ice-cold **RPMI complete (R10)** media, insert the syringe needle into the bone and flush out the bone marrow into a centrifuge tube on ice. Flush 2–3 times until the bones are completely white. Dissolve any clusters by pipetting.
 4. Centrifuge 1–2 times in **R10** media at 1,500 rpm for 5 minutes.
 5. Resuspend the cell pellet in **R10** and count viable cells using a hemocytometer and trypan blue.
 - ! Visit our website for [a detailed protocol on using a hemocytometer](#) to obtain a viable cell count
 6. Dilute the cells into 10 mL of **R10 + 20 ng/mL GM-CSF** and plate in petri dishes at a density of 2×10^6 viable cells per plate.
 7. Place plates in a 37°C incubator with 5% CO₂.
 8. Add an additional 10 mL of **R10 + 20 ng/mL GM-CSF** at day 3.
 - ! Add media very gently so as not to disturb the growing cells.
 9. Remove half of the media (10 mL) at day 6.

- ! Remove media very gently so as not to disturb the growing cells.
10. Briefly centrifuge the removed volume of media (as the DCs are only loosely adherent) and resuspend in 10 mL of fresh **R10 + 20 ng/mL GM-CSF** and add this back into the original culture.
- ! Cells can be harvested on day 8, or step 10 can be repeated to harvest cells on day 9 or 10.
11. Non-adherent and loosely adherent cells in the culture supernatant can be harvested by gentle washing with PBS, and then pooled for subsequent experiments.
- ! The majority of adherent cells will be macrophages, while those that are in suspension or loosely adherent will be dendritic cells (DCs) and F4/80+ macrophages. For a highly pure fraction, it is recommended to take measures to deplete F4/80+ cells and enrich the population for CD11c+ cells.
- ! Most of the macrophages in suspension will dually express F4/80+ and CD11c. The F4/80+ cells can be removed via positive selection with anti-F4/80 magnetic beads. After this step, CD11c+ DCs can be positively selected from the flow-through using anti-CD11c magnetic beads.
- ! Avoid using EDTA here as this will remove the adherent macrophages, diluting your DC samples.
12. The immature DC's can be used immediately after purification, or further cultured if the user wishes to obtain mature cells.
- ! DC's are loosely adherent and can mature simply by adhering to a plate, therefore a non-tissue culture treated 96-well u-bottom plate can be used to prevent premature maturation.

Purity assessment

It is recommended to perform flow cytometry on an aliquot of cells to check for purity. A purity check reagent can also be included to label cells still bound to magnetic beads.

Suggested markers

- [Anti-CD11c antibody \[N418\] \(ab33483\)](#)
 - This is also available labeled with Biotin, FITC, APC/Cy7, PE, PE/Cy7, PerCP/Cy5.5 and as low-endotoxin, azide-free
- [Anti-CD11b antibody \[EPR19373\] \(ab184307\)](#)
- [Anti-F4/80 antibody \[BM8\] \(ab16911\)](#) or [Anti-F4/80 antibody \[Cl:A3-1\] \(ab6640\)](#)
 - These antibodies are also available directly conjugated to FITC, PE or APC.

Independent testing

We asked an independent collaborator, Dr Karen Wozniak (Assistant Professor of Research, The University of Texas at San Antonio), to run our BMDC isolation protocol. Dr Wozniak was able to successfully purify a population of immature DC's, using the abcam reagents mentioned throughout the protocol. The data for these are outlined here.

Purity assessment

After culture with **GM-CSF** and purification using positive selection, cells were assessed for purity by flow cytometry. After gating on CD45+ cells, purity was determined based on the proportion of the population expressing CD11c (Figure 1).

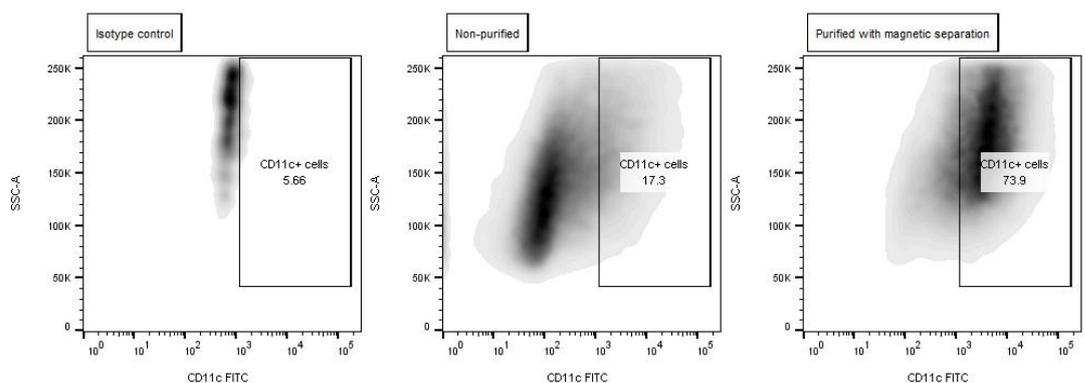


Figure 1. Following depletion of F4/80+ cells and positive selection of CD11c+ cells using magnetic beads, purity was checked using [anti-CD11c-biotin \(ab95765\)](#) conjugated to [streptavidin-FITC \(ab136201\)](#). Note: purity is greatly enriched when compared to the non-purified sample.

Stimulation assays

Immature DCs can be used immediately after purification if desired. However, users may wish to further culture their DC's with an antigen before adding T cells, in order to evaluate T cell proliferation in response to their antigen. Selected cytokines (eg [IL-2 \(ab9856\)](#), [IL-4 \(ab9729\)](#), [IFN-gamma \(ab9922\)](#), or LPS) can also be added in order to encourage increased antigen presentation by the DC's to the T cells.

To perform cell stimulations or treatments, plate the purified cells according the requirements of the downstream application.

- ! 2×10^5 cells per well in a 24-well plate is recommended if planning to run an ELISA. Alternatively, DC assays can be run in non-tissue culture treated 96-well u-bottom plates for a higher throughput.

Users may wish to check DC phenotype using flow cytometry, particularly if running T cell assays.

Suggested markers

Common DC markers, as indicated in our [immune cell markers guide to phenotyping](#).

- [Anti-MHC II antibody \[M5/114.15.2\]-PE \(ab93560\)](#)
- [Anti-CD80 antibody \[16-10A1\] \(ab106162\)](#) and [Anti-CD86 antibody \[GL-1\] \(ab119857\)](#)
 - This is not highly expressed in immature DCs but works in tandem to prime T cells
 - These antibodies are also available labeled with biotin, PE, FITC, PE/Cy5, or APC amongst other options

Phenotyping

Our independent collaborator used flow cytometry to assess the phenotype of mouse BMDCs immediately after purification (Figure 2) and following 24-hour culture of 2×10^5 cells per 100 μ L with 20 ng/mL [IL-4 \(ab9729\)](#) and 50 ng/mL LPS and LPS (Figure 3).

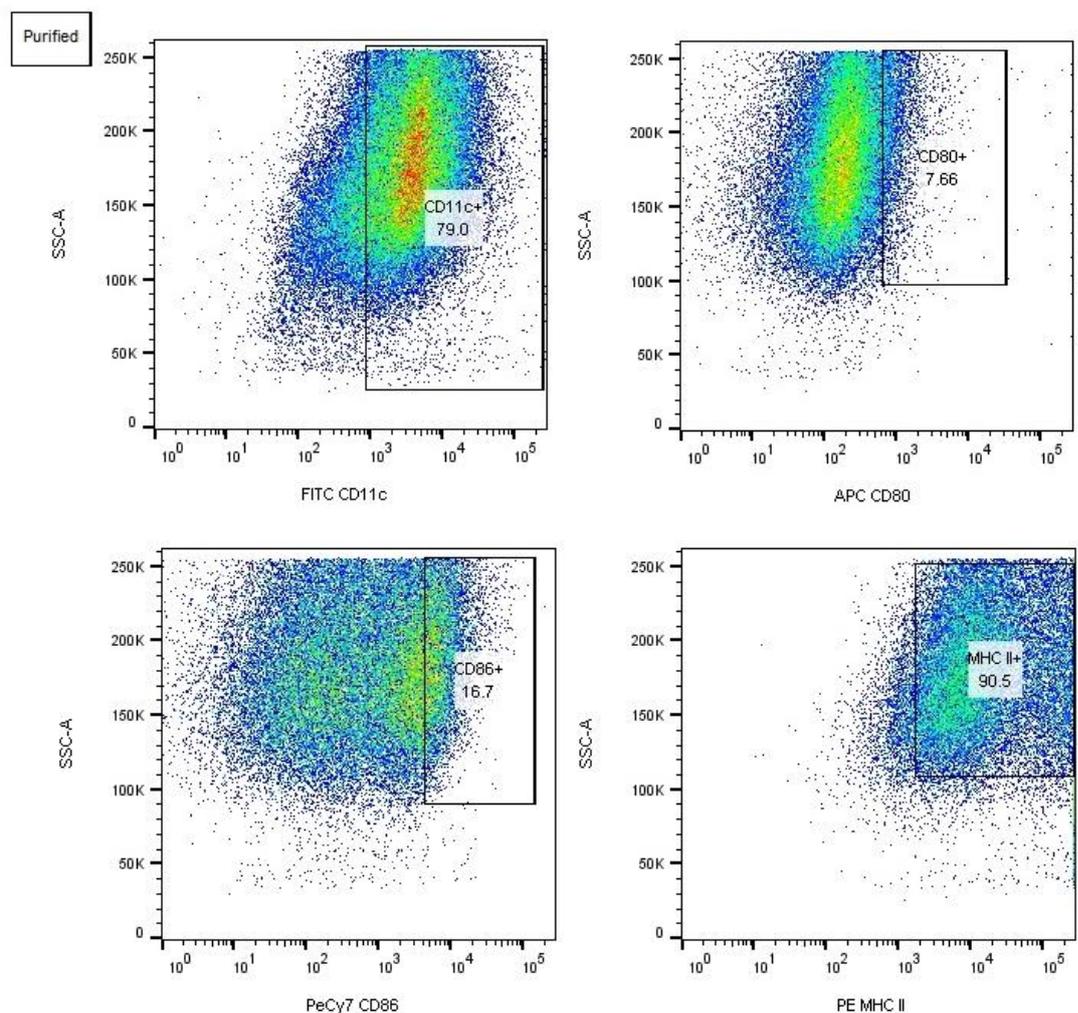


Figure 2. Purified mouse BMDCs were analyzed by flow cytometry immediately after purification using [CD11c-FITC \(ab95765\)](#), [CD80-APC \(ab25555\)](#), [CD86-PeCy7 \(ab81995\)](#), and [MHC II-PE \(ab93560\)](#).

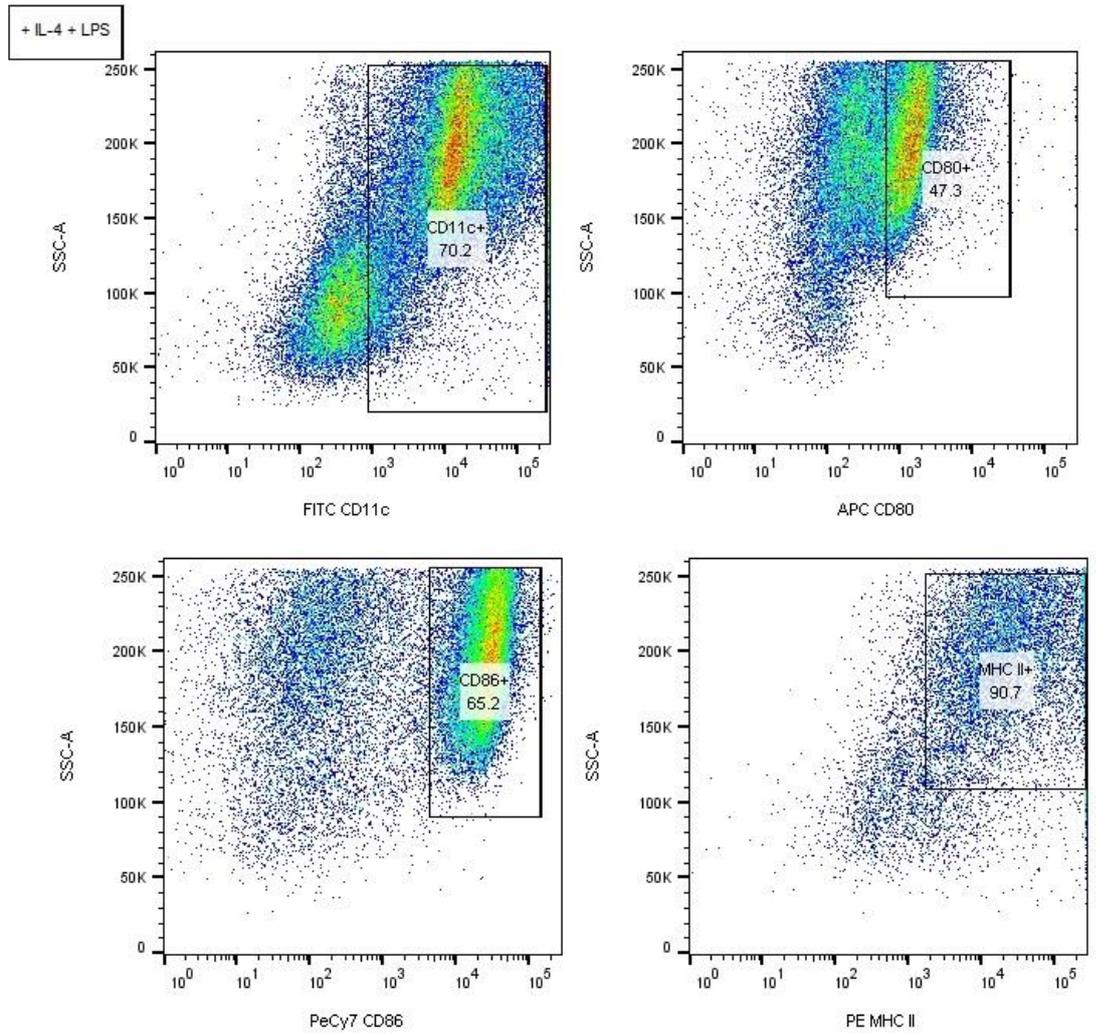


Figure 3. Purified mouse BMDCs were cultured with [IL-4 \(ab9729\)](#) and LPS for 24 hours before reassessment by flow cytometry using [CD11c-FITC \(ab95765\)](#), [CD80-APC \(ab25555\)](#), [CD86-PeCy7 \(ab81995\)](#), and [MHC II-PE \(ab93560\)](#).