

Generation of Mouse Bone Marrow-Derived Dendritic Cells (BM-DCs)

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[Abstract] Generating mouse dendritic cells from bone-marrow progenitor cells is a useful tool to study biological functions of mouse dendritic cells. Dendritic cells are one of the major populations of phagocytes able to activate both innate and adaptive immune cells.

Materials and Reagents

1. GM-CSF-transduced B16 cell line
2. HI FBS (EuroClone, catalog number: EC S0180L)
3. L-Glutamine (EuroClone, catalog number: EC B3000D)
4. Penicillin/streptomycin (EuroClone, catalog number: EC B3001D)
5. IMDM (EuroClone, catalog number: EC B2072L)
6. Beta-mercaptoethanol (Sigma-Aldrich, catalog number: M6250)
7. B16-GMCSF growth supernatant
8. Phosphate buffered saline (PBS) (EuroClone, catalog number: ECM9605AX)
9. EDTA
10. BMDCs culture medium/conditioned medium (see Recipes)

Equipment

1. Centrifuges 70 µm-wide cut-off cell strainer
2. Non-treated cell culture plates
3. Incubator (37 °C and 5% CO₂)
4. Fluorescence activated cell sorter (FACS)

Procedure

1. Flush mouse tibiae and femurs with ice-cold PBS through a 70 µm-wide cut-off cell strainer.
2. Centrifuge 5 min at 450 x g. Resuspend pelleted cells in conditioned medium (supplemented with 10% of growth supernatant of GM-CSF-transduced B16 cells).

3. Seed 7×10^6 cells in 100 x 20 mm non-treated cell culture plates in 10 ml of conditioned medium.
4. Incubate at 37 °C and 5% CO₂.
5. On day 4 and 7 add 5 ml of pre-warmed conditioned medium.
6. At day 8/9 the percentage of CD11c⁺ cells should be higher than 90% as measured by FACS analysis. BMDCs are then ready for experimental use.
7. Harvest the supernatant and gently wash the plate once with 5 ml of pre-warmed PBS.
8. Incubate 2 min with 5 ml of 2 mM EDTA at 37 °C and 5% CO₂.
9. Collect cells, wash once with PBS.
10. Centrifuge 5 min at 250 x g and resuspend pelleted cells in conditioned medium.

Recipes

1. BMDCs culture medium recipe (conditioned medium)
 - HI FBS - 10%
 - L-Gln - 2 mM
 - Penicillin/streptomycin - 50 U/ml
 - Beta-mercaptoethanol - 50 µM
 - B16-GMCSF growth supernatant - 10%
 - IMDM – to volume

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References

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