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Differentiation of bone marrow-derived macrophages

In 1 collection

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We use this protocol and it's working

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Abstract

This protocol describes the differentiation of bone marrow-derived macrophages using GM-CSF as a differentiation factor.

Materials

Plastic consumables:
- 20 ml syringe and 27G needles
- Falcon tubes
- 100 um cell strainer (eg. 130-098-463, Miltenyi Biotec)

Red blood cell lysis buffer, eg 00-4333-57, eBioscience

Media preparation:
DMEM (eg. 31966047, Gibco, ThermoFisher) +
- 10 % v/v heat-inactivated FCS
- 50 ng/ml murine GM-CSF (eg. 576306, BioLegend)

PBS/EDTA:
- PBS containing 2 mM EDTA

Troubleshooting

Differentiation

1 Collect femurs and clean bones of flesh.

2 Cut bones open on both ends and flush bone marrow into 50 ml Falcon tube with DMEM using a syringe.

3 Spin down cells at 300 x g for 5 min and resuspend pellet in red blood cell lysis buffer. Incubate for 10 min.

4 Add an equal amount of DMEM and filter cell suspension through a 100 um cell strainer.

5 Spin down cells at 300 x g for 5 min and resuspend cell pellet in DMEM containing 10 % FCS and 50 ng/ml murine GM-CSF.

6 Dispense cell suspension into 100mm Petri dishes containing a final volume of 10-12 ml of DMEM/ 10 % FCS/ 50 ng/ml murine GM-CSF.

7 Incubate cells at 37 °C, 5% CO_2.

8 At day 3 of differentiation: Add 5 ml of DMEM containing 10 % FCS and 50 ng/ml murine GM-CSF

9 At day 7 of differentiation: Harvest cells

Note

If there are many floating cells, cells can be split and differentiated further and initial cell densities can be decreased in the future.

Cell harvest

10 Remove medium and wash plates once in PBS.

11 Add 3 ml of PBS/EDTA to each Petri dish and place dishes in fridge for 10 to 15 min.

12 Harvest cells by flushing the surface of the Petri dish. Pool cells according to genotype and mouse ID.

13 Spin down cells at 300 x g for 5 min.

14 Resuspend cells in DMEM containing 10 % FCS and seed for experiments or freeze.

Freezing and thawing of BMDMs

15 To freeze cells, supplement DMEM/10% FCS with 10 % DMSO. Freeze over night at -80 °C and transfer to liquid nitrogen the following day.

16 To thaw cells, spin down cells to remove DMSO and resuspend pellet in DMEM/10% FCS containing 50 ng/ml murine GM-CSF. Plate cells accordingly and let recover for 24-48 hrs. On the day of the experiment, the GM-CSF can be omitted from the medium.