PROTOCOL EXCHANGE | *COMMUNITY CONTRIBUTED*

Generation of mouse bone marrow-derived macrophages (BM-MFs)

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Introduction

Protocol for generating mouse macrophages from bone-marrow progenitor cells used in our Nature paper.

Subject terms: Cell culture Tissue culture Immunological techniques Cell biology Developmental biology

Keywords: macrophage differentiation M-CSF

Reagents

M-CSF-transduced L929 cell line.

BMMFs culture medium recipe (conditioned medium):
HI FBS (EuroClone) – 10%
L-Gln (EuroClone) – 2mM
Penicillin/Streptomycin (EuroClone) – 50 U/ml
Beta-mercaptoethanol (EuroClone) – 50 microM
B16-GMCSF growth supernatant – 30%
IMDM (EuroClone) – to volume

Procedure

1.Flush mouse tibiae and femurs with ice-cold PBS through a 70 µm-wide cut-off cell strainer.
2.Centrifuge 5' at 1400 rpm. Resuspend pelleted cells in conditioned medium (supplemented with 30% of growth supernatant of M-CSF-transduced L929 cells).
3.Seed 7×10^6 cells in 100×20 mm non-treated cell culture plates in 10 ml of conditioned medium.
4.Incubate at 37 °C – 5% CO2.
5.Upon reaching confluence (approximately 7 days) split adhered cells and seed 5×10^6 cells in 100×20 mm in non-treated cell culture plates in 10 ml of conditioned medium.
6.BMMFs are ready for experimental use when the percentage of CD11b+ cells is higher than 90% as measured by FACS analysis.

Associated Publications

This protocol is related to the following articles:

- CD14 regulates the dendritic cell life cycle after LPS exposure through NFAT activation
  Ivan Zanoni, Renato Ostuni, Giusy Capuano, Maddalena Collini, Michele Caccia, Antonella Ellena Ronchi, Marcella Rocchetti, Francesca Mingozzi, Maria Foti, Giuseppe Chirico, Barbara Costa, Antonio Zaza, Paola Ricciardi-Castagnoli, and Francesca Granucci

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Competing financial interests

The authors declare no competing financial interests.

Readers' Comments

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Protocol Exchange ISSN 2043-0116