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 We use this protocol and it's working

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Ex vivo culture of SPMs

In 1 collection

Federico

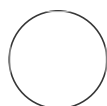
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ABSTRACT

Ex vivo culture of spleen derived macrophages.

ATTACHMENTS

[k4dpb48if.docx](#)

MATERIALS

Materials



RPMI 1640 Coris bioConcept Catalog #1-41F01-



DMEM High Glucose (45g/l) Coris bioConcept Catalog #1-26F03-

- 10% FBS
- 2mM L-Glutamine
- Penicillin-Streptomycin Pen 10'000 IU/ml
- amphotericin B (BioConcept 4-01F00-H)
- microglia BV2 cells from Elabscience (No.: EP-CL-0493)

Ex vivo culture of spleen derived macrophages

- 1 Dissect spleens from abdominal cavity and filter it through a 40- μ m nylon strainer.
- 2 Use red cell lysis buffer to remove red cells.
- 3 Then, obtain a single splenic cell suspension. Culture cells in Roswell Park Memorial Institute (RPMI) medium 1640 RPMI 1640 (BioConcept 1-41F01-I) supplemented with 10% FBS, 2 millimolar (mM) L-Glutamine and antimicrobials (Penicillin-Streptomycin Pen 10'000 IU/ml Strep 10 mg/mL and amphotericin B (250 μ g/mL BioConcept).
- 4 Culture mouse microglia BV2 cells from Elabscience (No.: EP-CL-0493) in parallel for each spleen culture preparation as controls.
- 5 Briefly, maintain BV2 cells in Roswell Park Memorial Institute (RPMI) medium 1640 supplemented (BioConcept 1-41F01-I) with 10% FBS (FBS-02-0500), 2 millimolar (mM) L-Glutamine 5-10K50-H) and antimicrobials (Penicillin-Streptomycin Pen 10'000 IU/ml Strep 10 mg/mL and amphotericin B (250 μ g/mL) (BioConcept 4-01F00-H).
- 6 Spleen macrophages (SPMs) differentiate into the M1 phenotype after stimulation with LPS (100 ng/ml) \pm IFN- γ (10 ng/mL).
- 7 For BV2 stimulation, replace RPMI by Dulbecco's Modified Eagle Medium (DMEM) High Glucose. (BioConcept, 1-26F03-I).