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Ex vivo culture of SPMs

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Protocol status: Working
We use this protocol and it's working

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Abstract

Ex vivo culture of spleen derived macrophages.

Attachments

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13KB

Materials

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RPMI 1640 **Coris bioConcept Catalog #**1-41F01-I

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

- 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)

Troubleshooting

Ex vivo culture of spleen derived macrophages

1 Dissect spleens from abdominal cavity and filter it through a 40-µm 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, [M] 2 millimolar (mM) L-Glutamine and antimicrobials (Penicillin-Streptomycin Pen 10'000 IU/ml Strep 10 mg/mL and amphotericin B (250 µg/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), [M] 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 µg/mL) (BioConcept 4-01F00-H).

6 Spleen macrophages (SPMs) differentiate into the M1 phenotype after stimulation with LPS (100 ng/ml) ± IFN-γ (10 ng/mL).

7 For BV2 stimulation, replace RPMI by Dulbecco's Modified Eagle Medium (DMEM) High Glucose. (BioConcept, 1-26F03-I).