L929 CONDITIONED MEDIUM (LCM)

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BACKGROUND

Macrophage colony stimulating factors (M-CSF) are secreted by L929 cells (ATCC) and promote bone marrow progenitors to differentiate into a heterogeneous population of bone marrow derived macrophages (BMDM) expressing various myeloid surface markers (CD11b, F4/80, BM8, CD31, CD68, CD11c, Ly6C, GR1)[1]

EQUIPMENT

- L929 fibroblasts
- Water bath
- DMEM – 10
- Tissue culture flasks
- Incubator
- Trypsin [Invitrogen]
- Falcon tubes
- 0.45 µm filter

PROTOCOL

1. Thaw L929 fibroblasts stored in -140°C in a 37°C degree water bath for 2 minutes.
2. Pipette thawed L929 gently into a 50 mL falcon tube containing 20 mL of warm DMEM – 10.
3. Centrifuge the cell suspension at 1500 rpm for 5 minutes.
4. Resuspend the cell pellet in 5 mL DMEM – 10 media and pipette into a T25 tissue culture flask.
5. Incubate at 37°C, 5% CO_2 overnight to grow to 70% Confluency.
6. Cells were lifted with 5 mL trypsin and pipetted into a 50 mL falcon tube containing 20 mL DMEM – 10 media. Cell suspension was centrifuged at 1500 rpm for 5 minutes.
7. Cell pellet was re-suspended in 15 mL DMEM-10 media and transferred to a T75 flask.
8. Incubate at 37°C, 5% CO_2overnight to grow to 70% Confluency.
9. Cells were lifted with 10 mL trypsin and pipetted into a 50 mL falcon tube containing 20 mL DMEM – 10 media. Cell suspension was centrifuged at 1500 rpm for 5 minutes.
10. Cell pellet was re-suspended in 25 mL DMEM-10 media and transferred to a T150 flask.

11. Cells were cultured for 2 days, reaching a confluency of 90% and split 1:5 into a new T175 flask. This process was repeated until 50 flasks were obtained.
12. Split L929 cells 1:5 into a new T175 flask containing 45 mL DMEM -10 and culture for 10 days at 37°C, 5% CO_2.
13. The cell culture media was collected and centrifuged at 3000 rpm, 4°C. the media containing M-CSF was then filtered through a 0.45 µm filter and frozen at -27°C in 50 mL aliquots.

LINKS AND REFERENCES

1. Bender AT, Ostenson CL, Giordano D, Beavo JA. Differentiation of human monocytes in vitro with granulocyte-macrophage colony-stimulating factor and macrophage colony-stimulating factor produces distinct changes in cGMP phosphodiesterase expression. *Cell Signal*. 2004;16(3):365-374.