Mouse BMDM

Rabia Khan

Abstract

Citation: Rabia Khan Mouse BMDM. **protocols.io**
dx.doi.org/10.17504/protocols.io.gcqbsvw
Published: 07 Nov 2016

Before start

Aseptic Removal of Femurs

Small beaker of 70% EtOH

Scissors

Dissection blades

Tweezers

Ice

50 mL Falcon with 25 mL cold phenol red free media or PBS on ice for each strain used.

Dissection board and pins (3)

Under tissue culture hood

3ml syringe

20G/ 26 G needle

*RPMI media (RPMI 10%FBS, 1% pen/strep, glutamine 2mM)

*RPMI 10%FBS **without** Pen/Strep – *PHENOL FREE FOR LDH*

*Prepare day before

6 well plates

50ml tubes

Dissection blades

Sterile petris

Protocol

Step 1.
Put 2 mL media per well in 6-wells plate (1 well per 2 marrows or per animal)

Step 2.
Clean femur as much as possible in a petri with a little bit of phenol red free media or PBS (the most important part is the middle)

Step 3.
Cut off both ends of the femur to expose the marrow

Step 4.
Fill syringe up with the 2ml of media, insert into marrow and flush into the 6 well plate until the bone is clean (goes from pink to white).

Step 5.
Pass the media through the syringe 1-2 times (Start with 20G, then 26G)

Step 6.
Transfer the cells to a 50 ml tube

Step 7.
Prepare RBC lysis solution (proportions in cell culture cabinet) – Or purchase from Sigma

Step 8.
Add 5ml per marrow.

Step 9.
Place on ice for 10min excatly.10. Centrifuge for 10min at 4°C 400g (RCF, or 1200 rpm)

Step 10.
Discard supernatant

Step 11.
Add 5ml CSF and 12ml of RPMI Medium (total volume is 15 ml) – Do not let cells dry, add medium immediately. (Or 9ml RMPI medium and 3ml CSF)

Step 12.
Transfer the 15 mL cell suspension to a 100 mm polystyrene tissue culture petris (PrimariaTM, Becton Dickinson Labware) for 24 hrs

Step 13.
Transfer non-adherent cells after 24 hrs to fresh polystyrene petris or non adherent flasks (Green - Fisher). Cultures are grown for 6 days with 15% (v/v) L-929 cell-conditioned medium as a source of M-CSF. Add fresh CSF every 3 days. (15% of 15ml – (about 3ml)a. The batch made by Kyoko and Rabi ais at 30% , so use 5ml.

Step 14.
After 6 days, collect the cell with a policeman (cell scraper 25 cm, Sarstedt #83,1830).Centrifuged for 10min 400 x g. Discard supernatant and resuspend pellet in 2ml of RPMI 10%FBS without Pen/Strep

Phenol free for LDH. Flush media through 25, 27, and 30 G needles to separate aggregates.

Step 15.
Count cells to final concentration of 1 x 106 cells/ml. To count cells, dilute 1/5 in Turks. Aliquot 500 µL (5x105 cellules) per well in 24-wells plate, 100ul for 96 well plate.

Step 16.
Incubate o/n, 5% CO2 before infection.

Step 17.