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

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Harvesting and culturing of peritoneal macrophages and ex-vivo stimulation

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ABSTRACT

Harvesting and culturing of peritoneal macrophages and ex-vivo stimulation with IFNy

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Prior to sac

1 3 days prior to sac

- 30minutes to an hour prior to Thioglycolate injection, give slow-release buprenorphine SubQ as a proactive measure against any pain or discomfort
- Intraperitoneal inject (IP) mice with 1mL of sterilized, 3% thioglycolate solution per mouse and return to home cage for 3 days.

Prep

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- Set large centrifuge to 4 degrees fast temp
- Large container of ice
- 1 x 50mL falcon per mouse
- 1 x 15mL falcon per mouse
- 1 x 10mL syringe plus 27G needle – filled with cold RPMI media
- 1 x 10mL syringe plus 25G needle – empty
- Surgical tools: 2 x hemostat, 2 x forceps, 1 x artery scissors (ball tip), 1 x surgical scissors
- 70% ethanol spray bottle
- Turn on laminar flow hood and spray down with 70% ethanol, line with puppy pad/s
- 1 or 2 polystyrene blocks wrapped in puppy pad
- Transfer pipettes
- 1 x 70uM nylon filter per mouse
- HBSS-/- at room temp
- 25mL stereological strippettes
- Pipette aid
- Plating media – RPMI (containing L-Glut) supplemented with 1 x pen strep and 10% FBS. Place amount needed in bead bath to warm ready (for nucleofection, warm in falcon and plate in the incubator to acclimate)

Methods

3

- On the third day after thioglycolate injection, sacrifice mice by cervical dislocation
- Spray the abdomen with a 70% ethanol-water solution to disinfect and prevent cut fur from spreading
- The skin of the abdomen should be split along the midline with a pair of artery scissors and forceps, taking care to avoid puncturing or cutting the abdominal cavity
- Once the cavity has been revealed, use a 10mL syringe with a 27G needle to inject 10mLs of cold

RPMI media into the peritoneal cavity

- Gently massage the sides of the abdomen to aid in cell suspension
- Use a 25G needle on a 10mL syringe to withdraw as much fluid as possible. Care must be taken to avoid puncturing any organs or blood vessels to avoid contaminating the suspension with erythrocytes as much as possible. Place aspirated fluid into 15mL falcon
- When no more fluid is able to be aspirated, cut open the abdominal cavity, again taking care to avoid cutting any organs or blood vessels.
- Use a plastic transfer pipette to aspirate any of the remaining RPMI-thioglycolate fluid in the cavity
- Collect the fluid into the same 15mL falcon and place on ice. The peritoneal fluid of each mouse will be kept separate.
- Once collected, place 70uM nylon filter onto 50mL falcon and pre-wet with 5mL of HBSS-/-. Filter aspirated fluid through the filter to form a single cell suspension. Wash filter twice with 5mL of HBSS-/-.
- Spin tubes at 400 x g for 5 minutes at 4°C.
- Remove and discarded supernatant, and resuspend the resulting pellet of cells in 3mL RPMI supplemented with 10% FBS and 1x Pen-Strep (pre-warmed in bead bath or incubator).
- Count cells using trypan-blue exclusion and then plate at an appropriate density for the assay.
- Incubate cells at at 37°C, 5% CO_2 for a minimum of two hours, after which the majority of the macrophages will have adhered to the surface of the dish. Two generous washes with sterile PBS will remove non-adherent cells, leaving only attached macrophages behind. Replace with new, pre-warmed RPMI supplemented with 10% FBS and 1x Pen-Strep, and return to the incubator or continue with assay
- For ex vivo stimulation, include 100nG/mL of IFNy and incubate cells for 18 hours and continue with downstream assay