**Murine Peritoneal Macrophages Isolation**

**REAGENTS & SUPPLIES**

* 4% Thioglycollate broth (Sigma #T-9032) (4% Thioglycollate in PBS, 0.22μm filtration)
* Surgical scissors, Surgical forceps
* 10% FBS + DMEM + L/G + P/S media
* 10mL Syringe, 30G\*1/2 needle (for media injection), 25G-26G needle (for media harvest)
* 50mL polypropylene centrifuge tubes

**EXPERIMENT**

< Day0 >

-Inject 1mL of 4% thioglycollate broth to peritoneum of each mouse

< Day3-4 >

-Sacrifice mouse by CO2, spray 70% ethanol onto the mouse and open the exterior skin of mouse

-Inject 8mL of DMEM media to peritoneum of mouse using 27Gneedle, shake well, and then harvest media from peritoneum of hold the mouse upside down using the 22G needle

-Remove needle from syringe and harvest pooled peritoneal media to 50-ml polypropylene centrifuge tubes on ice.

*-*Re-inject 8ml of DMEM and harvest again.

-1000rpm 10min 4°C centrifugation

-Discard supernatant, add new DMEM, and re-suspend cell pellet gently

-Cell count using hemocytometer

-Adjust to appropriate cell concentration in each plate, incubate at 37°C, 5% CO2 cell incubator