



Institutional Animal Care and Use Committee™

Title: Collection of Thioglycollate-Elicited Peritoneal Macrophages

SOP Number: 066

Purpose: This document outlines the procedure for collection of Thioglycollate-Elicited Peritoneal Macrophages in mice, which is an important technique to study innate immune responses and macrophage metabolism.

Recommended supplies:

1. Brewer thioglycollate broth prepared a head of time in the lab:
 - a. 3-4 % wt/vol thioglycollate medium (Sigma) in ddH₂O, autoclaved, prepared from dehydrated thioglycollate medium and sterile saline water. Thioglycollate solution needs to be wrapped with aluminum foil to avoid light and be placed at room temperature to age for several weeks until it turns to brown in color.
2. Sterile needle and syringe
 - a. 23-30 gauge needle
 - b. 5-10 mL syringe
3. 70% ethanol
4. Ca²⁺- and Mg²⁺-free Hanks' balanced salt solution
5. Autoclaved forceps and blunt-end scissors
6. 50 mL-sterile tubes
7. Ice

Animal Procedure:

1. The mouse is given an intraperitoneal injection of the prepared Brewer thioglycollate broth (3 %), which induces an inflammatory reaction and recruits macrophages to the peritoneal cavity.
 - a. Dose volume for mice ≥ 2 months of age is 2-3 ml and is 1-1.5 ml for mice that are < 2 months.
2. Mice are monitored as described below.
3. The mouse is euthanized following an IACUC approved animal protocol 1 to 4 days post-injection, depending on the mouse strain and the number of cells needed.

Potential Adverse Effects:

1. Thioglycollate intraperitoneal injection: injection site pain.
2. Improper IP injection: solution is injected into abdominal organs (bowel, bladder) instead of peritoneum resulting in peritonitis or damage to internal organs.
3. Treated mice will be monitored daily for signs of discomfort, including hunched posture, ruffled fur, lethargy and lack of movement around the cage. Body condition score (BCS) index will also be used to evaluate the welfare of mice. If the animals show signs of discomfort or a BCS ≤ 2 , they will be euthanized.

Macrophage Collection Procedure:

1. Sterile Ca²⁺- and Mg²⁺-free Hanks' balanced salt solution and empty 50 mL-sterile tubes are placed on ice.

2. The euthanized mouse is fixed in dorsal recumbency on a dissecting board with pins.
3. The abdominal skin is sterilized with 70% ethanol.
4. Using the forceps, the skin is pulled up in the inguinal region and an incision is made using scissors. DO NOT cut the peritoneum/abdominal cavity.
5. The mouse's skin is bluntly dissected from the peritoneum/abdominal wall.
6. The skin is cut in a "U" shape and pulled away from the peritoneum.
7. The elicited peritoneal cells are collected by peritoneal lavage with 10 mL of cold Ca^{2+} - and Mg^{2+} -free Hanks' balanced salt solution three times from the peritoneal cavity. Be cautious not to puncture any internal organs, especially the intestines.
8. Peritoneal fluid is collected into 50 mL-sterile tubes and immediately put on ice.