

Standard Operating Procedure Whole Mouse Perfusion

I. Purpose

This document provides the protocol for whole mouse perfusion using 0.9% saline solution. This protocol can be used to clear blood cells from organs and tissues so that tissue- and organ-specific analyses are not confounded by the presence of blood cells. This protocol is adapted from usual whole animal perfusion techniques so that blood can be collected for cell sorting purposes. Saline solution is used in attempt to minimize any unintended physiological interactions between tissues and the perfusion solution.

II. Materials

Lab supplies and reagents required for this SOP include the following:

- Cell culture grade saline solution (0.9% NaCl)
- Collection tubes with EDTA (e.g., microtainer)
- Saf-T Wing blood collection & infusion set
 - Needle size 25G x 3/4" w/6" tube
- Syringes (x2)
 - 10 mL for PND21 mouse OR
 - 20 mL for 5 month-old and 10-month-old mice
 - 1 mL for blood collection
- Needle sized 26G1/2
- Surgical scissors
- Surgical forceps
- Ethanol (70%)
- CO₂ tank with adjustable pressure gauges
- Small clear plastic enclosed container specially designed for mouse euthanasia
- Lab bench absorptive pads

III. Procedure

1) Preparation

- a. Label collection tube with mouse ID, sex, and age
- b. Clean all surgical equipment using soap and water, followed by sterilization with ethanol
- c. Place absorptive pad on lab bench in the area in which perfusion and blood collection will be performed.
- d. Fill perfusion syringe with saline, connect butterfly needle to the end of the saline syringe, and eject a small amount of saline to ensure that there are no air bubbles in the tube connected to the butterfly needle.
 - i. Select an appropriate size syringe based on mouse size. See section II above for suggestions.
- e. Attach needle to 1mL syringe for blood collection

2) CO₂ euthanasia

- a. Place mouse in euthanasia container and perform CO₂ euthanasia according to approved animal protocol

- b. Once animal appears to be euthanized, perform a toe pinch to ensure that the animal is non-responsive
- 3) Perform pneumothorax
 - a. Use ethanol to wet the antral side of the animal
 - b. Locate and grasp sternum with forceps
 - c. Make a lateral incision just under the sternum using surgical scissors. Make the incision as large as necessary to be able to clearly see the diaphragm.
 - d. Re-position forceps to grasp the sternum bone and cartilage and lift up. Cut liver and gall bladder away from the diaphragm as necessary.
 - e. Make an incision in both sides of the diaphragm, taking care not to cut or puncture the heart.
 - f. Ensure that both lungs are collapsed following diaphragm incision.
- 4) Open chest cavity
 - a. Grasp the sternum with forceps.
 - b. Use scissors to cut up the left and right sides of the chest, cutting through the ribcage. Take care to avoid cutting through the heart and major blood vessels.
 - c. Cut pericardial fat so that the heart is free and does not stick to the chest wall.
- 5) Blood collection
 - a. Insert 1mL syringe with needle into the right ventricle and slowly draw blood, taking care not to draw so quickly that the heart immediately collapses. The right ventricle will collapse towards the end of collection, but it is important that it collapses over the course of 30-45 seconds.
 - b. Withdraw syringe from ventricle and eject blood into collection tube containing EDTA.
 - c. Immediately place tubes on ice or at 4C
 - d. Process blood within a few hours of collection time
 - e. Refer to blood processing protocol
- 6) Perfusion
 - a. Insert Saf-T wing needle with syringe containing saline into the left ventricle and hold it there. If needle slips out, attempt to re-insert the needle into the same hole.
 - b. Slowly use the syringe to inject a small amount of saline into the left ventricle. The right ventricle should begin to inflate again.
 - c. Use surgical scissors to cut a small nick in the right atria. This will allow the blood and saline mixture to leak into the chest cavity as the mouse is perfused.
 - d. Continue to slowly inject saline while keeping the heart neither under nor over inflated.
 - i. Injection rate should be approximately 1mL/minute
 1. Perfusion will be more complete and effective if the heart is still beating throughout the perfusion process. Injecting saline at a slow rate should help to keep the heart beating as long as possible. A good rule of thumb is to make sure that

the heart is neither over- or under-inflated. If the heart stops beating, either stop or start injecting saline and then wait a few seconds to see if it starts again.

- e. Perfusion is complete once the fluid being collected in the empty syringe runs clear.
- f. Turn mouse over onto the absorptive pad so that the blood and saline mixture that was contained in the chest cavity drains onto the pad. Some of the saline-blood mixture will also likely leak out onto the pad during the perfusion process.