

SOP_MTL-1.5 Terminal Blood Collection from Mice

- A. Purpose:** To provide techniques and instructions for terminal blood collection from mice and the process used to obtain plasma or serum.
- B. Scope:** The techniques described in this SOP are used to obtain blood samples via the inferior vena cava from mice to produce whole blood, plasma, or serum specimens.

C. Definitions:

EtOH: Ethanol

PDX: Patient-derived xenograft

RCF: Relative Centrifugal Force

TG: Transplant generation

Transplant generation: The number of times that PDX tissue has been transplanted from mouse to mouse with the purpose of maintaining an actively growing PDX model

D. Materials and Reagents:

Name	Quantity	Cat number	Sterility status for use
0.5 M EDTA, pH 8	50 µL	15575-020, ThermoFisher	Non-sterile
1 mL Syringe	1	14-823-434, Fisher scientific	Sterile
1.7 mL Microcentrifuge tubes	2-5/ mouse	NC9818380, Denville	Sterile
1000 µL Pipette tips (+pipette)	1-3	1000 µL: 05-403-18, Eppendorf	Sterile
200 µL Pipette tips (+pipette)	3	200 µL: 05-403-19, Eppendorf	Sterile
23g Hypodermic Needles	6	14-826-6B, Fisher scientific	Sterile
25g Hypodermic Needles	3	14-826AA, Fisher scientific	Sterile
50 mL Conical tubes	1	14-959-49A, Fisher scientific	Sterile
70% Ethanol spray bottle	1	LC222102, Fisher scientific	Non-sterile
Body bag	1	BCM Animal Facility	Non-sterile
Centrifuge	1	5424/5424 Eppendorf	Non-sterile
Cotton tip applicator	3	22-363-168, Fisher scientific	Non-sterile
Euthanasia Box	1	NA	Non-sterile

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Isoflurane	1	BCM Animal Facility	Sterile
Kimtech wipes	2-3	06-666A, KC	Non-sterile
Labels	As needed	B-490, Brady	Non-sterile
Paper towels	1	10714-002, VWR	Non-sterile
Regular forceps	1	RS-5139, Roboz surgical	Sterile
Scissors	1	RS-5880, Roboz surgical	Sterile
Securline marker (ethanol resistant)	1	14-905-30, Fisher scientific	Non-sterile
Sharps disposal container	1	Provided by environmental safety department	NA
Styrofoam board	1	NA	Non-sterile

E. References:

SOP_MTL-1.4 Tumor Tissue Excision for PDX Maintenance

SOP_MTL-1.11 Harvest Sheets

F. Procedures:

Note: This protocol describes blood collection from a euthanized animal. This generally allows collection of 400-700 µL of whole blood. If more blood is needed, the procedure can be performed by placing the mouse under a surgical plan of anesthesia so that full exsanguination is done on a live mouse which allows for continual blood flow during collection.

1. Whole Blood/Plasma Collection Preparation:

- 1.1. For each animal, take a 1 mL syringe and pull up 1 mL of 0.5 M EDTA. Place a 25g needle on the syringe, remove bubbles, and push the EDTA through the needle back into the original bottle. This will coat the inside of the needle and syringe with EDTA to help prevent clotting during the collection. Also, this will leave EDTA in the hub of the needle.
- 1.2. Label a 1.7 mL tube, using an ethanol resistant marker, with the PDX model and mouse identifier for each animal. This tube will be used for the whole blood collected from the mouse. (VIAL1)
- 1.3. Add 50 µL of EDTA to each tube.
- 1.4. Print labels with the PDX model, TG, mouse identifier, date, and PLASMA for each animal. Place the labels on 1.7 mL tubes. These tubes will be used to store the aliquots of plasma. The number needed per animal will depend on the size of the aliquots that are requested, and the amount of plasma obtained. (VIAL2)

2. Serum Collection Preparation:

- 2.1. Place a 25g needle on a 1 mL syringe for each mouse.
- 2.2. Label a 1.7 mL tube with the PDX model and mouse identifier for each animal using an ethanol resistant marker. This tube will be used for the whole blood collected from the mouse. (VIAL1)
- 2.3. Print labels with the PDX model, TG, mouse identifier, date, and SERUM for each animal. Place the labels on 1.7 mL tubes. These tubes will be used to store the aliquots of serum. The number needed

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per animal will depend on the size of the aliquots that are requested, and the amount of serum obtained. (VIAL2)

3. Prepare a Euthanasia Box:
 - 3.1. Put KIMTECH wipes in a 50 mL conical tube.
 - 3.2. Place the euthanasia box in the chemical hood and pour isoflurane in the 50 mL tube until the wipes are saturated. Pour off any extra isoflurane back into the isoflurane bottle.
 - 3.3. Place the tube in the bottom of the box and ensure no isoflurane leaks out. The mouse should not come in direct contact with the isoflurane.
- 3.4. Make sure the lid is securely placed on the box.
 4. Prepare workstation by placing an absorbent pad on the benchtop and gather supplies needed for the blood collection: scissors, forceps, prepared syringes, cotton swabs, and gauze pads.
 5. Select a styrofoam board and place six 23G needles along the edges. Place a napkin on the foam board to place the mouse on.
 6. Place a mouse inside the euthanasia box. Watch it closely and as soon as it takes its last breath remove it from the box.
 7. Place the mouse on the styrofoam board ventral side up. Secure the limbs to the board with 23G needles. Verify that the mouse is not breathing.
 8. Spray the entire ventral surface of the mouse with 70% EtOH to wet the hair down and prevent it from getting into the body cavity of the mouse.
 9. Use forceps to tent the skin at the lower abdomen and use scissors to make a midline incision from between the #5 nipples to the base of the neck. From the lowest incision point, make an inverted Y incision by cutting distally toward each hind limb. Be sure not to cut through any large vessels in the fat pad while making the incisions.
 10. Gently separate the skin from the peritoneum using a dry cotton-tipped applicator on each side of the body. Make sure the peritoneum is pushed back toward the midline of the body and not stretched out on the flaps of skin. Fold each skin flap out and pin to the board using a needle.
 11. Use forceps to hold the peritoneum above the bladder and make a small incision. Extend the incision cranially up each side of the peritoneum laterally to each of the major vessels that are visible. This will make a U-shaped flap that can be folded back towards the head. Reference image in Appendix H2.
 12. Use a cotton tipped swab to move the fat and intestines to the technician's right side, which will expose the inferior vena cava. Be sure to position the membrane over the vena cava so that you have a clear view of the vessel.
 13. Take the appropriately prepared syringe and place the needle bevel side up on top of the vena cava. Do not touch the tip of the needle anywhere else prior to this step. Slowly push forward until the vena cava is punctured. Push the needle into the vessel until about 1 cm is inserted but be careful to stay parallel to the vessel so that the needle doesn't puncture the back wall.
 14. Slowly pull back on the plunger to start withdrawing the blood. If the vein starts to collapse, pause and let it refill. If the vessel suction shuts, use a cotton tip applicator to pump the chest cavity or gently wiggle the needle back and forth. This usually allows more blood to flow into the vessel.
 - 14.1. Do not pull back on the plunger too quickly as mouse blood hemolyzes very easily.
 - 14.2. Perform this step quickly and efficiently to avoid hemolysis and preliminary clotting. If the draw becomes difficult, do not waste too much time trying to get more blood, this will only cause more hemolysis.

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15. Once all the blood is withdrawn, remove the needle from the inferior vena cava, pull the plunger back to remove remaining blood from the needle, remove the needle and place it in a sharps container.
16. Remove the blood from the syringe into VIAL1 by slowly pushing the plunger of the syringe. Do not do this step too quickly as shear forces can cause hemolysis. Do NOT push the blood back through the needle as the shear forces will cause hemolysis.
 - 16.1. If collecting plasma, gently mix the blood with the EDTA in the tube.
17. To process whole blood:
 - 17.1. Keep the blood on ice until processing if being used the same day. If it is to be frozen, make the appropriate size aliquots (VIAL2), label, and store at -80°C.
18. To process plasma:
 - 18.1. Place the tube in the centrifuge as quickly as possible and balance the centrifuge.
 - 18.2. Centrifuge the blood at 2000 RCF for 10 minutes.
19. To process serum:
 - 19.1. Allow the blood to clot for 30 minutes at room temperature on the benchtop.
 - 19.2. Centrifuge the blood at 1300 RCF for 15 minutes.
20. If any other organs or tissues need to be collected from the animal, follow the adequate SOPs (for tumor excision reference *SOP_MTL-1.4 Tumor Tissue Excision for PDX Maintenance*).
21. After centrifugation, pipette the plasma/serum off the top. Be careful not to disturb the clot at the bottom of the tube and do not aspirate any of the clot or blood cells.
22. Place the aliquoted amount of plasma/serum into VIAL2. Repeat as needed until all plasma/serum has been aliquoted.
23. If a plasma sample has hemolysis, that needs to be noted by indicating the color level (slightly pink, moderately pink, very pink, red). Add this information to the comments on the Harvest Sheet.
24. Plasma can be stored at -20°C for short-term storage (<3 months). Store at -80°C for long-term storage.
25. Serum should be stored at -80°C.
26. Clean tools with gauze pad and 70% EtOH and place in the dirty tool bin.
27. Place mouse carcass in the body bag, wrap up, tape, and place in the -80°C freezer.
28. Clean up bench area and tools according to lab guidelines.

G. Revisions log:

Version	Revision Date	Section Revised	Notes
1	03.30.2021	All	SOP created

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H. Appendix:

1 Harvest Date: 09/23/20

2 Harvested By: Lacey Dobrolecki

3 PDX 4664

4 TG R3TG4

5 Transplant Date 07/28/20

6 Fragments for Transplantation 10

7	8	9	10	11	12	13	14	15	16	17
Mouse	FFPE Block	Snap Frozen	STR	LDEV (spleen)	Snap Location (List -80°C storage location)	Viable	Viable Location (List deep freeze storage location)	Blood	Other Collaborators	Tumor Size
A	2300	2	1	1		7		John		10.2x9.8
B	2301	2				7		Hari		9.7x8.9
C	2302	1				10			Tissue to Fuqua Lab	9.5x9.2

18 Data entered into OpenSpecimen

H.1 Harvest sheet.



H.2 Incision line image.