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Plasma Isolation Standard Operating Procedure

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We use this protocol and it's working

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Disclaimer

The authors have nothing to disclose

Abstract

This document describes the process for labeling plasma tubes, collecting plasma samples, storing plasma samples, and creating a sample manifest for reverse phase protein microarray (RPPM) biomarker analytical platform. Examples are *Italicized*. Attention items are in **bold**.

Attachments

Agoston_Lab_SAMPLE_M...
57KB

Appendix 1.pdf
51KB

Guidelines

- Collect blood directly from the left ventricle using a 25 gauge needle + 1mL syringe. A blood sample of 200-300 µL should be enough to yield 80-150 µL of plasma.
- Remove the needle from the syringe before putting whole blood into a BD Microtainer collection tube for plasma preparation.
- Immediately after the blood sample has been taken, gently invert the tube 180° and back 8-10 times to ensure proper mixing of anticoagulant with blood.
- Centrifuge at 1100 g for 10 minutes at 4°C.
- Following centrifugation, maintain the tubes at 2-8°C while handling.
- Transfer the resulting supernatant into 3 clearly labeled cryovials.
- Promptly flash-freeze tubes in dry ice and store at -80°C or lower in pre-designated boxes.

Materials

- Centrifuge
- BD Microtainer PPT™ tubes (Catalog no. 365974-1; purple closure; specification: K2EDTA)
- Sarstedt Screw cap micro-tube, 1.5 mL, PCR Performance Tested, Low protein-binding (Sarstedt Catalog no. 72.703.600)
- Refrigerated microcentrifuge (Labnet Prism R; Catalog no. C2500-R, or similar item)

Troubleshooting

Safety warnings

Attention items are written in **Bold**. Correct mixing of the EDTA tube is extremely important to avoid microclotting.

Ethics statement

This protocol follows institutional regulations consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines for reporting animal research. Experimental procedures involving animals comply with protocol No. 30608, approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, Riverside, School of Medicine, and protocol No. 25046 approved by IACUC at Indiana University.

Before start

Turn on the centrifuge and set the temperature to 4°C before sample preparation (lid must be closed for centrifuge to cool down).

Scope

The scope of this SOP includes:

- Solutions required to successfully complete vessel painting, perfusion, and fixation
- Equipment setup and solution recipes
- The euthanasia procedure
- The surgical procedure
- Post-fixation methods
- Attention items are written in Italicized Bold

Responsibilities

- SOP Author: responsible for production of the SOP and its described procedures
- SOP Owner: responsible for reviewing and approving changes to the SOP
- Staff: responsible for adhering to SOP guidelines
- NO CHANGES to this SOP are authorized without the explicit approval of the SOP Owner (See Appendix 1)

Preparation

1 Turn on the centrifuge and set the temperature to 4°C before sample preparation (lid must be closed for centrifuge to cool down).

2 Blood should be collected in 1 collection tube per mouse and labeled manually with a regular permanent marker. **Use only BD Microtainer PPT™ tubes (Catalog no. 365974-1; purple closure; specification: K2EDTA) for blood collection and plasma processing.**
Example of a properly labeled collection tube for one sample with a SoftMouse-compatible RapID: MV1925

3 Given the limited space for writing on cryovials, they must, at a minimum, be labeled with a Softmouse System ID (SID#), RapID#, AO#, or IU ID, as well as the project name. Use a digital printer to prepare the tube labels. We are currently using a Zebra printer (ZD421T) with custom polyester labels. **Use only the following tubes for long-term plasma storage: Sarstedt Screw cap micro-tube, 1.5 mL, PCR Performance Tested, Low protein-binding. (Sarstedt Catalog no. 72.703.600).**
Example of 3 printed tube labels for one sample: (RapIDProject NameAliquot#)
MV1925 TBI-ADRD #A MV1925 TBI-ADRD #B MV1925 TBI-ADRD #C

4 Once samples have been collected and flash-frozen in dry ice, place them in three separate plasma sample boxes (each box holds 81 samples, 9 rows by 9 columns). Store one aliquot per box for a total of 3 boxes per sample. Each box must be labeled on both the top and the front to help you find it easily. If more than 81 samples will be collected for a given project, also number the box.
Example of 3 boxes generated for one set of samples: (Note that each box contains a specific aliquot)
TBI-ADRD (IU or UCR), BOX #1_Aliquot #A
TBI-ADRD (IU or UCR), BOX #1_Aliquot #B
TBI-ADRD (IU or UCR), BOX #1_Aliquot #C

5 Each box has an associated electronic **manifest spreadsheet that must be updated and saved each time a sample is added**. The associated manifest should be derived from this “map” layout. Only one manifest per project is used. Follow the instructions at the top of the manifest spreadsheet. See Attachments for a sample of the manifest.
Note: For digital records, ensure the most recent copy of this manifest is in the appropriate folder within the TBI-ADRD project folder. Each box should hold one aliquot per sample. The example shown contains different samples using RapID as the identifier for a box called "TBI-ADRD rCHI (UCR), Box #1_Aliquots #A." All of the samples stored here hold aliquot #A for a given sample, so there should be two more boxes with the same "map" layout containing aliquots #B, and #C. The RapID shown in this example can be substituted with a SoftMouse SID#, AO#, IU ID, or a Tattoo ID, etc...

TBI-ADRD rCHI (UCR), BOX #1_Aliquots #A

		COLUMN								
		1	2	3	4	5	6	7	8	9
ROW	A	MV1925 #A	XE1119 #A	HO1864 #A	XE1112 #A	XE1118 #A	HO8440 #A	XE1108 #A	SD1308 #A	SE6886 #A
	B	MV4111 #A	HO1384 #A	SD1307 #A	WR9265 #A	XG0120 #A	CK9051 #A	CK9047 #A	9071BQ #A	5060BS #A
	C	3490BS #A	0470BQ #A	3491BS #A	SE6886 #A	XE3405 #A	SD1303 #A			
	D									
	E									
	F									
	G									
	H									
	I									

Plasma Isolation Procedure

6 Collect blood directly from the left ventricle using a 25ga needle + 1mL syringe (If you are doing vessel painting, get blood BEFORE injecting the dye). A blood sample of 200-300 µL should be enough to yield 80-150 µL of plasma.

7 **Remove the needle from the syringe before putting whole blood into a BD Microtainer collection tube for plasma preparation**. [Note: Pushing blood through the needle will cause hemolysis and compromise plasma sample]. The BD Microtainer PPT™ tube (Catalog no. 365974-1; purple closure; specification: K2EDTA) separates undiluted plasma from whole blood for molecular diagnostic tests.

8 Immediately after the blood sample has been taken, gently invert the tube 180° and back 8-10 times to ensure proper mixing of anticoagulant with blood. **Correct mixing of the EDTA tube is extremely important to avoid microclotting**.

9 Centrifuge at 1100 g for 10 minutes at 4°C.

10 Following centrifugation, maintain the tubes at 2-8°C while handling.

11 Transfer the resulting supernatant into 3 clearly labeled cryovials, taking care to leave behind the cell pellet and any white buffy coat that may be present.

Order of Operation: First transfer all supernatant to the tube with aliquot #C, homogenize gently by pipetting in and out, then transfer 30 µL to Tube #A, and 30 µL to Tube #B.

There will be 3 tubes per animal with volumes divided as follows:

Tube with aliquot #A = 30 µL
Tube with aliquot #B = 30 µL
Tube with aliquot #C = Remaining sample- Volume varies

12 Promptly flash-freeze tubes in dry ice and store at -80°C or lower in pre-designated boxes. Freeze-thaw cycles must be avoided.