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JAX-Sen: Placenta collection, processing, and shipment protocol

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Cellular Senescence Net...



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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol outlines the procedures for the collection, processing, and shipment of mouse placenta samples.

Safety warnings

- ⚠ Follow all institutional guidelines for animal handling and euthanasia. Use personal protective equipment (PPE) when handling reagents and samples.

Reagents and Materials:

1 Reagents

Buffer MAC's buffer - Used for preserving placenta samples

Fixative 10% Neutral Buffered Formalin (NBF) - Used for tissue fixation

Buffer RNAlater - Preserves RNA in samples

Buffer PBS

Materials

Dissecting scissors (Sharp, stainless steel)

Petri dishes

Vials (5 mL and scintillation vials)

Ice (For wet ice preparation)

Cold packs (For sample shipping)

Procedure:

2 Collection:

2.1 Euthanize mice via cervical dislocation.

a. Record start time

2.2 Open the female's abdomen carefully as to not damage any fetuses.

a. Euthanize fetuses via decapitation if they are older than E15

2.3 Remove the hind left limb from one animal per litter.

2.4 Keep in 1x PBS until ready to take a image for limb staging

2.5 Separate individual yolk sac containing fetus and embryos

2.6 Remove any un-needed tissue (such as yolk sac and uterine wall) from placenta. There will be a layer of tissue on the side on the placenta connecting to the umbilical cord that



will need to be removed. Similar looking layer of tissue on the other side is part of the placenta and should not be removed.

a. Step 6 to 9 can either be done in order of embryo or all steps for all embryos at once

2.7 Take appropriate measurements in mm

a. Diameter of the placenta

b. Crown to rump length (CRL) of embryo

2.8 Process Placenta

	A	B	C	D
	MAC's buffer	place placenta in 5 mL vial filled with the buffer	Ensure that the placenta is completely submerged	Place tube in wet ice
	10% NBF	place placenta is scintillation vial filled with FFPE (10% NBF)	Ensure that the placenta is completely submerged	
	RNA later	place placenta in 5 mL vial filled with the buffer	Ensure that the placenta is completely submerged	Place tube in wet ice

2.9 Take piece of the embryo (ideally tail) for genotyping and another piece as back-up. Place on wet ice.

2.10 Record time when the last sample was collected

2.11 Clean tools and proceed to next animal

3 Limb Staging:

3.1 Cut the left hind limb from the embryo

3.2 Put the limb in a Petri dish with PBS (palm side down)



- 3.3 Take an image of the limb and save it as a JPEG.
- 3.4 Open <https://limbstaging.embl.es/> in a browser.
- 3.5 Upload the image and use 'pan and zoom mode' so that the limb fills the whole box
- 3.6 Trace the limb as accurate as possible
- 3.7 Press 'Stage Embryo' and record the result.

4 **Sample Storage:**

	A	B	C
	MACs Buffer	Keep at 4C (wet ice)	Ship same day on cold packs
	RNA Later	Keep at 4C (wet ice)	Ship same day on cold packs.
	FFPE	Keep at 4C (wet ice)	Transfer to a shaker at room temperature to fix for 24 hours. Then transfer and submit samples while keeping them at 4C and RNase free
	Embedded Placentas	Keep at 4C/RNase free	until ready for shipping

5 **Sample Shipping:**

- 5.1 Ship via same day shipping to the Robson Lab on cold packs to keep the samples at 4C.