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Collection of Mammalian Embryonic Palate Tissue

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Works for me

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ABSTRACT

This protocol will guide you through the process of extracting embryos from adult mice and the manipulation of embryos in order to obtain palatal tissue.

GUIDELINES

This protocol was designed using CD-1 mice acquired from Charles River Laboratory. Mouse were bred per CD-1 Breeding Protocol published by MRF.

MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
50ml Conical Tubes, Green Cap, Sterile, 25/Bag	CT788-GS.SIZE.1PK	Bio Basic Inc.
Sodium azide	DB0613.SIZE.100g	Bio Basic Inc.
BupH Phosphate Buffered Saline Packs	PI28372	Thermo Fisher Scientific
Low Protein Binding Collection Tubes (1.5 mL)	90410	Thermo Fisher Scientific
Fisherbrand™ High Precision Dissecting Micro Scissors	08-953-1B	Fisher Scientific
Nikon SMZ18 Stereoscope	View	Nikon Instruments
Lab Armor Beads	A1254301	Thermo Fisher Scientific
Ice Bucket	M16807-1104	

MATERIALS TEXT

Fill ice bucket with lab armor beads and cool to $\delta -20^{\circ}\text{C}$

Make at least ml of Volume Percent NaN3 PBS

Aliquot ml of the PBS solution into 50ml conical tubes and cool to $\delta 4^{\circ}\text{C}$

SAFETY WARNINGS

Clean all working areas and equipment before and after use.

BEFORE STARTING

Breed mice per CD-1 Breeding Protocol.

Prepare euthanasia chamber and gather supplies prior to proceeding.

Preparation

- 1 Euthanize pregnant mouse via primary and secondary modes of IACUC approved euthanasia
CO2 gas at 2L/min until 1 minute after last gasp.
Cervical separation

Embryo Collection

- 2 Using scissors, create an abdominal incision from the genitalia to the rib cage.
- 3 Locate and lift both strands of embryos so they are clear of all organs.
- 4 Free embryos by cutting the tissues connecting anteriorly at the kidney and posterior at the base of the uterine horn.
- 5 Transfer the embryos to the 50ml tube containing PBS on ice.
Embryos should remain on ice until all sample collection is complete.
- 6 Place adult mouse in ziplock bag and place in Animal Room freezer

Processing

- 7 In a dish containing PBS, remove each embryo from the uterine strand using micro scissors.
- 8 Cut open the amniotic sacs and cut the umbilical cords to free the embryos from the placenta.
- 9 Remove the heads of the embryos and transfer to a dish of clean PBS.

Palate Collection

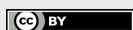
- 10 Transfer an embryo head to a clean sorting dish and place under stereoscope at 1.75X zoom.
- 11 Remove tongue and mandibular tissues.

12 Excise palatal shelves via cutting the tissues connecting the palatal shelves to the nasal palatal and maxillary processes.

13 Transfer palate tissue to 1.5ml centrifuge tube on ice.

Preservation

14 Preserve palate tissue by freezing at -80°C in PBS solution.



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