

Jul 24, 2019

Collection of Mammalian Embryonic Palate Tissue

DOI

dx.doi.org/10.17504/protocols.io.5tag6ie

Matthew Fox¹

¹Texas Tech University



Matthew Fox

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DOI: dx.doi.org/10.17504/protocols.io.5tag6ie

Protocol Citation: Matthew Fox 2019. Collection of Mammalian Embryonic Palate Tissue. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.5tag6ie>

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Protocol status: Working

We use this protocol and it's working

Created: July 24, 2019

Last Modified: July 24, 2019

Protocol Integer ID: 26178

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

MATERIALS

✕ 50ml Conical Tubes, Green Cap, Sterile, 25/Bag **Bio Basic Inc. Catalog #CT788-GS.SIZE.1PK**

✕ Sodium azide **Bio Basic Inc. Catalog #DB0613.SIZE.100g**

✕ BupH Phosphate Buffered Saline Packs **Thermo Fisher Scientific Catalog #PI28372**

✕ Low Protein Binding Collection Tubes (1.5 mL) **Thermo Fisher Scientific Catalog #90410**

✕ Fisherbrand™ High Precision Dissecting Micro Scissors **Fisher Scientific Catalog #08-953-1B**

✕ Nikon SMZ18 Stereoscope **Nikon Corporation**

✕ Lab Armor Beads **Thermo Fisher Scientific Catalog #A1254301**

✕ Ice Bucket **Catalog #M16807-1104**

Fill ice bucket with lab armor beads and cool to **🌡 -20 °C**

Make at least **🧴 500 mL** of **[M] 0.02 % volume NaN3** **PBS**

Aliquot **🧴 35 mL** of the PBS solution into 50ml conical tubes and cool to **🌡 4 °C**

Safety warnings

❗ Clean all working areas and equipment before and after use.

Before start

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.