Isolation and Processing of Embryonic and Postnatal Brains

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We use this protocol and it's working

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ABSTRACT

This protocol describes:
-Harvesting embryos from females from embryonic day 10 [e10] to embryonic day 18 [e18]
-Brain isolation of embryonic (e10-e18) and postnatal brains (p0-p30)
-Processing and Freezing of embryonic and postnatal brains

Harvest embryos from female mouse from embryonic day 1...

1 Place pregnant female in an anesthetic chamber for 2 minutes

2 Euthanize female using cervical dislocation

3 Grip the abdominal wall with forceps and make incisions along the region to expose the abdomen

4 Cut away the uterus with scissors

5 Place the uterus in a petri dish filled with 1x phosphate-buffered solution [1x PBS]

6 Carefully poke the amniotic sac right next to the placenta to expose the embryo.

7 The embryo will slide out of the sac, do not handle the head of the embryo with the forceps

8 Behead at the neck of the embryo and collect a tail clip into its corresponding falcon. Each falcon should be filled with 4% paraformaldehyde [4% PFA]

9 Repeat to the remaining embryos in the uterus

10 Embryonic heads are left in 4% PFA overnight for fixation

Brain isolation of embryonic and postnatal brains

11 Leave embryonic heads in 4% PFA for one night, and postnatal heads in 4% PFA for two nights

12 When fixation is complete, transfer the brain from 4% PFA into 1X-PBS

13 Place petri dish under a dissecting microscope [from Leica Microsystems]

14 Place head in the petri dish

15 The head is held down and stabilized by inserting the forceps into the eyes

16 Push the forceps inwards, making a medial cut

17 Using fine forceps, make horizontal cuts along the jaw. Do this to remove everything about the tongue, leaving behind the brain and skull only

18 Tilt the head on its side and use forceps to pull away between the skin and the skull to expose the brain. Do this while rotating the head as needed to remove the skin and skull

19 When the brain is exposed, slide forceps along the base of the skull and pinch off the brain to remove it from the head

Processing and Freezing of embryonic and postnatal brains

20 When the brain is isolated, place the brain in 30% Sucrose prepared in 1X PBS

21 Wait until the brain sinks. For embryonic brains, this takes 3-16 hours while postnatal brains take 24-35 hours

22 When the brains have sunk, they are ready to be frozen

23 Fill a bucket with powdered dry ice

24 Place Isopentane bottle and the brain's respective falcon on top of the dry ice. Make sure they are secured within the dry ice

25 Pour the brain into a petri dish

26 Gently scoop the brain from the petri dish and place it on top of a thickly folded paper towel

27 Roll a KimTech Dry wipe and use it to gently roll the brain along the paper towel. This will ensure that the brain is dry

28 Once the brain is dried, slowly drop the brain into the Isopentane solution

29 Leave embryonic brains for 15-20 minutes and postnatal brains for 25-30 minutes in the isopentane bottle for proper freezing

30 Once the freezing is done, gently scoop the brain out of the isopentane bottle and gently run the spoon over a KimTech dry wipe to dry the liquid. Make sure to work quickly in this step, so the brain does not thaw.

31 Transfer the brain into its respective falcon, and place the falcon in -80C.

32 Brains frozen with Isopentane can be stored in the -80C indefinitely.