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Isolation and Processing of Embryonic and Postnatal Brains

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Protocol status: Working

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

Troubleshooting

Harvest embryos from female mouse from embryonic day 10 (e10) to embryonic day 18 (e18)

- 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.