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Protocol status: Working
We use this protocol and it's working

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mouse brain storage and sectioning

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ABSTRACT

detailed protocol to receive mouse brain tissue from US to Australia including storage and sectioning

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Storage and sectioning of mouse brain tissue

1 Archive mice brains for storage

1.1 Sample arrive in the delivery room (4°C)

1.2 Move samples from the delivery room to PC2 laboratory.

1.3 Cross check tissue ID with shipping log

1.4 Inspect for floating tissue in 30% sucrose and discoloration.

1.5 Inspect for floating tissue in 30% sucrose and discoloration.

1.6 Prepare labelled (date of arrival and tissue ID) 50ml falcon tubes filled with new sucrose solution (30% sucrose, 0.02% sodium azide).

1.7 Pour old sucrose out and gently place tissue into the new falcon tubes.

1.8 Store tubes in 4°C on shaker overnigh.

Sucrose solution/PB buffer/Cryoprotectant solution for mouse...

2 30% sucrose solution

2.1 Dissolve 300g of sucrose in 300mL of distilled water

2.2 Add 100mL of 10x PBS

2.3 Adjust final volume to 1L with distilled water.

2.4 Add 2mL of 10% sodium azide to solution.

2.5 Mix well until solution is homogenous and store in 4°C

3 1X Phosphate Buffer (1M)

3.1 In 1.8L of dH2O, add 38.7g of NaH2PO4.H2Oand 101.9g of Na2HPO4.

3.2 Mix solution until it is homogenous and store in 4°C.

4 Cryoprotectant Solution for Mouse Brain (1L)

4.1 Add 300mL of glycerol, 300mL of ethylene glycol, 100mL 1xPhosphate Buffer, and 300mL of dH_2O

4.2 Mix solution overnight on the stirrer at RT.

4.3 Store solution at 4°C

tissue cutting and storage

5 Mice brain sectioning and storage

5.1 Tubes are filled with cryoprotectant to 90% of volume: 8 tubes for ST sections, 8 tubes for SN sections, 1 tube for PF sections. One tube without cryoprotectant is prepared for leftover tissues.

5.2 Create a rectangle mould using metal plates to fit the mouse brain.

5.3 Pour Clear O.C.T Compound Embedding Medium to the mould up to ¼ of the volume. Ensure that the medium is evenly distributed.

5.4 Place the mould filled with embedding medium inside the Epredia™ CryoStar™ NX50 chamber and use the quick freeze option to quickly freeze the medium

5.5 Move sample from the cool room to RT

5.6 Retrieve the mould with frozen embedding medium and pour more medium up to ½ of the volume

5.7 Gently place the mouse brain to the mould using forceps, submerging it in embedding medium. Adjust the orientation as needed

5.8 Slowly pour more embedding medium on top of the mouse brain until it is completely submerged in embedding medium

5.9 Place the mould back inside the Epredia™ CryoStar™ NX50 chamber and quickly freeze it

6 Lift the frozen mould from the chamber and mark the front orientation of the brain by drawing a line on the frozen embedding mould using a sharpie

6.1 Pour a 1cm diameter dollop on the circular cutting plate

6.2 Release the mould and place the frozen tissue on the cutting plate, front orientation up

6.3 Place the tissue and cutting plate into the chamber, quickly freezing it

6.4 Place the frozen tissue and cutting plate to the cutting table. Adjust the orientation so that the mould is parallel to the cutting blade.

6.5 Cut PF with 20um of thickness and place it in the PF vial.

6.6 Cut ST with 20um of thickness and sequentially place them in 8 vials, repeat the process until SN is reached

6.7 Change section thickness to 30um to cut SN and sequentially place them in 8 vials, repeat the process until the cerebellum is reached.

6.8 Release the frozen cerebellum and cutting plate from the cutting table

6.9 Remove the frozen cerebellum from the cutting plate and place it into the vial for leftover.

6.10 Vials are then placed into designated boxes and stored in -20°C