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Brain Clarification for Neuromelanin visualisation by OPT

In 2 collections

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

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Disclaimer

The **protocols.io** team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Abstract

Protocol for the clarification of whole mouse brains to allow neuromelanin visualisation by Optical Projection Tomography

Troubleshooting

Brain processing

1 Mice are transcardially perfused in 4% PFA and postfixed in the same fixative overnight

2 After rinsing several times in PBS, brains are embedded in 1% low melting point agarose in water

3 Brains are dehydrated in 100% methanol covered in aluminum foil to protect the specimen from light; change the methanol three times during the next 24 h or until the specimen is completely dehydrated

4 Once dehydrated the brains are incubated during 4 hours shaking in 66% dichloromethane (DCM) /33% methanol at room temperature

5 The brains are incubated with shaking in 100% DCM 30 minutes twice to complete the delipidation process

6 The brains are chemically cleared with BABB (a combination of 1 part of benzyl alcohol and 2 parts of benzyl benzoate)

7 The brains are subsequently immersed in a BABB-filled chamber for Optical projection tomography (OPT) imaging

Brain imaging

8 The brains are scanned with transmission light with three different filters (a cyan fluorescent protein -CFP-filter: emission 460-500nm, a green fluorescent protein -GFP-filter: emission 500-550nm and a DSR filter: emission >610nm) using an OPT imaging system mounted on a Leica MZ 16 FA microscope

9 The brain images are visualised using the open source software Fiji