# Delipidation Protocol

This protocol is used to remove lipid content from fixed brain tissue via dehydration, to facilitate later section-based or whole-mount tissue staining and clearing.

Note: The optimal duration for each step depends on the sample. Example durations and buffer volumes are provided for single hemispheres and whole adult mouse brains.

**Guidelines**:

- For brain hemisphere: use 2mL Eppendorf tube and ~1.4-1.6mL solution for each wash.
- For whole brain: use 5mL Eppendorf tube and ~4.5mL solution for each wash.
- ***Optional Tip***: During 100% MeOH and 100% DCM wash steps, move the tissue sample into an extra tube to minimize solution carry over and prevent sample from drying in the air.

IMPORTANT SAFETY NOTICE:

1) MeOH is volatile and flammable. Handle it with care and follow safety regulations.
2) DCM is volatile and an irritant. Use it inside a non-recirculating fume hood. Wear double nitrile gloves to handle the extraction process. Discard gloves immediately when contaminated with DCM.

***AdipoClear+ Delipidation for Sectioning***

| (A) Hemisphere | (B) Whole Brain | Explanation |
|---|---|---|
| Total Length: 13 hrs | Total Length: 3 days | |
| 1. Dehydrate the sample by washing up gradient of MeOH in B1n (0%, 20%, 40%, 60%, and 80% MeOH): Shake gently at RT, 30min for each step. | 1. Dehydrate the sample by washing up gradient of MeOH in B1n (0%, 20%, 40%, 60%, and 80% MeOH): Shake gently at RT, 1hr for each step. | To remove relatively soluble lipids in cellular and organelle membranes. |
| 2. Wash in 100% MeOH: shake at RT, 30min x2. | 2. Wash in 100% MeOH: shake at RT, 1hr x2. | To remove hydrophobic lipids in myelin and lipid storage. |
| 3. Wash in 100% DCM: shake at RT, 1hr. | 3. Wash in 100% DCM: shake at RT, o/n. | |
| 4. Wash in 100% MeOH: shake at RT, 30min, 45min, 1hr. | 4. Wash in 100% MeOH: shake at RT, 1hr, 1.5hr, 2hr. | |
| 5. Rehydrate with reversed MeOH/B1n gradient (from 80% to 20%): shake at RT, 30min each. | 5. Rehydrate with reversed MeOH/B1n gradient (from 80% to 20%): shake at RT, 1hr each. | To rehydrate back to conditions required for immunostaining. |
| 6. Wash in B1n buffer: shake at RT, 2hr. | 7. Wash in B1n buffer: shake at RT, o/n. | |

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| 7. Wash in PBS w/$NaN_3$: shake at 37°C, 30min, 1hr, o/n. | 9. Wash in PBS w/$NaN_3$: shake at 37°C, 1hr, 2hr, 4hr, o/n. | |
| Samples can then be stored in PBS w/$NaN_3$ at 4°C for a few weeks. Fill the tube to top with media to reduce air oxidation. | | |