

Large- scale tissue clearing while preserving endogenous fluorescence

This method is adapted from (Schwarz et al. 2015; Stefaniuk et al. 2016; Klingberg et al. 2017) by Wouter Masselink (Wouter.masselink@imp.ac.at)

This protocol is divided into two parts, dehydration and clearing. For both dehydration and clearing a variety of solutions can be used. For dehydration both 1-Propanol (1P) and Tert-Butylalcohol (TBA) can be used, while for clearing both BABB (a 1:2 mixture of Benzyl-Alcohol and Benzyl-Benzoate) and Ethyl Cinnamate (ECi) can be used. All solutions except for the Ethyl-cinnamate should have their pH set between 9 and 10 using Tri-ethylamine.

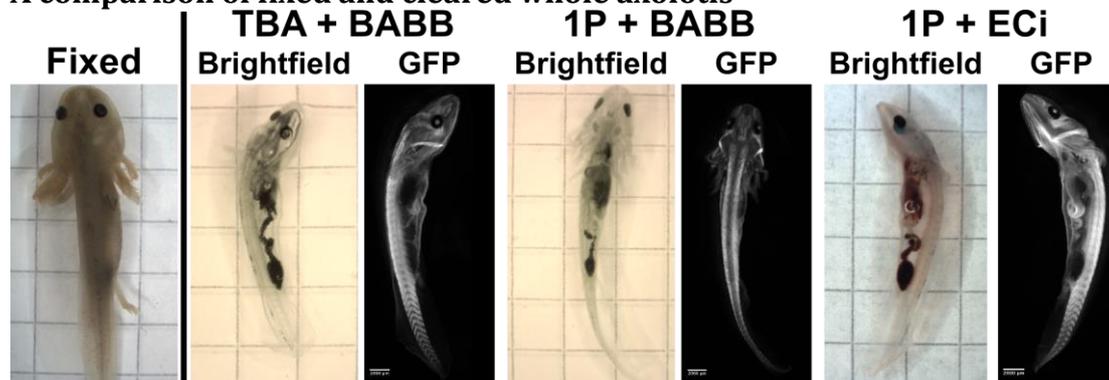
!!! Please use all necessary precautions and perform all steps in a fume-hood !!!

1. Fix tissue as per normal (4% PFA, Formaldehyde etc...)
2. Wash tissue 2x 10 minutes in PBS (pH9-10)
3. Dehydrate in 30% TBA or 1P in PBS 24 hours (pH9-10)
4. Dehydrate in 50% TBA or 1P in PBS 24 hours (pH9-10)
5. Dehydrate in 70% TBA or 1P in PBS 24 hours (pH9-10)
6. Dehydrate in 80% TBA or 1P in PBS 24 hours (pH9-10)
7. Dehydrate in 96% TBA or 1P in PBS 24 hours (pH9-10)
8. Dehydrate in 100% TBA or 1P in PBS 24 hours (pH9-10)
9. transfer to BABB (pH9-10) or ECi clearing solution.
10. Clear for 1-3 days change clearing medium every 24 hours.
11. Store tissue in clearing medium at 4C.

Some thoughts:

- Dehydration steps can be shortened on a tissue-by-tissue basis. Once a tissue sinks to the bottom, it should have equilibrated and further dehydration can be done.
- The choice between TBA and 1P is ultimately up to the user, I did not observe any difference between the two. Considering TBA is significantly more expensive than 1P, I would recommend the use of 1P over TBA.
- The choice between BABB and ECi is based on similar criteria. Both efficiently clear while preserving the fluorescence. BABB is much cheaper than ECi and should thus be considered. Before you choose one or the other please consider that BABB dissolves most plastics and is not particularly nice. On the other hand ECi has a similar refractive index but is considered food-grade and does not dissolve most plastics. ECi is a solid at 2-8C, which might negatively affect the morphology of the sample when in long-term storage.

A comparison of fixed and cleared whole axolotls



Reagents

1-Propanol (Sigma-Aldrich: 279544)
Tert-Butyl alcohol (Sigma-Aldrich: 471712)
Benzyl alcohol (Sigma-Aldrich: 305197)
Benzyl benzoate (Sigma-Aldrich: B6630)
Ethyl-cinnamate (Sigma-Aldrich: 112372)
Triethylamine (Sigma-Aldrich: T0886)
PBS

References

- Klingberg, A. et al., 2017. Fully Automated Evaluation of Total Glomerular Number and Capillary Tuft Size in Nephritic Kidneys Using Lightsheet Microscopy. *Journal of the American Society of Nephrology : JASN*, 28(2), pp.452–459.
- Schwarz, M.K. et al., 2015. Fluorescent-protein stabilization and high-resolution imaging of cleared, intact mouse brains. W. H. Merigan, ed. *PLoS ONE*, 10(5), p.e0124650.
- Stefaniuk, M. et al., 2016. Light-sheet microscopy imaging of a whole cleared rat brain with Thy1-GFP transgene. *Scientific reports*, 6(1), p.28209.