The effects of DeepLabel™ Antibody Staining Kit on antibody penetration in diverse organs after tissue clearing with the X-CLARITY™

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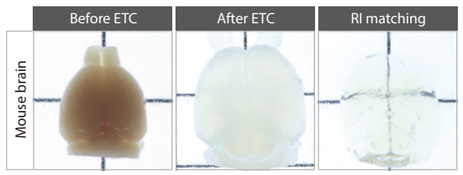
INTRODUCTION

- Tissues are inherently three dimensional in nature, which makes imaging intact tissues a necessity for a more complete study into the relationship between structure and function and the system-level study of cellular mechanisms.
- Tissue clearing techniques have allowed biologists to acquire high-resolution volumetric images without the need to reduce samples to thin serial sections.
- The X-CLARITY™ is a collection of systems and reagents for tissue clearing based on CLARITY. CLARITY is an aqueous-based tissue clearing technique in which a sample is infused with hydrogel monomers and then heated to initiate radical polymerization, covalently linking the biomolecules in the tissue sample to a sturdy hydrogel network. Lipids are broken up through electrophoresis in the presence of ionic detergents, resulting in a transparent tissue-hydrogel hybrid that is chemically accessible for multiple rounds of antibody labeling and imaging.
- DeepLabel™ Antibody Staining Kit enhances antibody penetration into large clarified tissues and contains buffers for permeabilization and antibody labeling.

Materials & methods

- All animal experimental procedures were conducted in accordance with KBRI IACUC guidelines. Female mice and rats (8 weeks) were anaesthetized with Avertin (250 mg/kg, Sigma) and perfused transcardially with PBS, followed by 4% PFA. Brains, spinal cords, and sciatic nerves were extracted and further fixed in 4% PFA at 4°C for 24 hr.
- The tissue samples were then incubated X-CLARITY Hydrogel Solution Kit (C1310X) for 24 hr at 4°C. The hydrogel was polymerized in the X-CLARITY Polymerization System (C20001) at 37°C for 3 hr at -90 kPa.



RI = Refractive index

- After polymerization, tissues were cleared in the X-CLARITY Tissue Clearing System II (C30001) at 1.2 A, 60 V, 37°C, and 100 rpm.
- For immunolabeling, samples were labeled in TH (1:100, Abcam), Collagen IV (1:100, SouthernBiotech), α-SMA (1:100, Abcam), β-tubulin (1:100, Abcam), GFAP (1:100, Dako), and Parvalbumin (1:100, Abcam). Samples were labeled using PBST (control) or DeepLabel Antibody Staining Kit (C33001).

DeepLabel™ Antibody Staining Kit increases permeabilization compared to conventional methods.

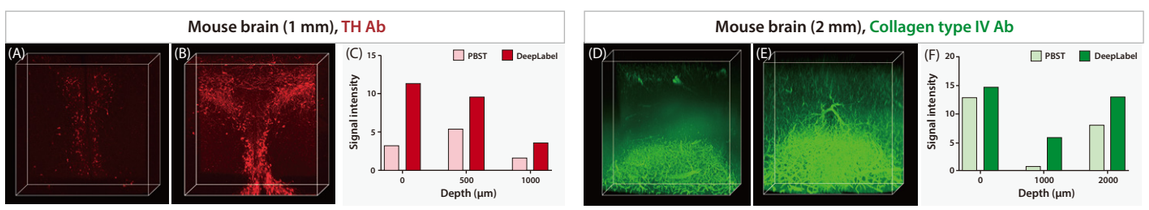


Figure 1. Comparison of antibody staining efficiency with and without DeepLabel. DeepLabel Antibody Staining Kit enhances antibody penetration and site-specific binding in thick, cleared tissues for robust and efficient labeling. 1 mm (A-C) and 2 mm (D-F) mouse brain slices were labeled for TH (red) and Collagen type IV (green), respectively, and the samples labeled in DeepLabel had markedly better antibody distribution and low background for excellent signal-to-noise ratio.

DeepLabel™ Antibody Staining Kit enables site-specific labeling in diverse organs.

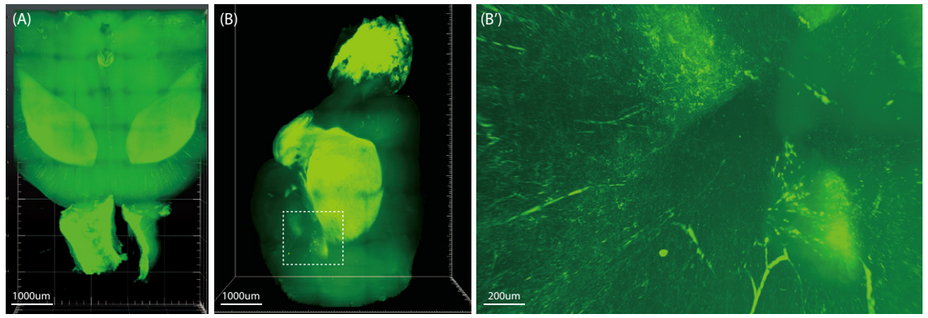


Figure 2. TH signal distribution in a whole and half brain stained with DeepLabel. DeepLabel improved the penetration of TH antibodies, which resulted in whole (A) and half (B) brains being homogenously stained with high signal-to-background after 1 day permeabilization and 3 days each in primary and secondary antibody solutions. A magnified image (B') shows the highly specific labeling of dopaminergic neurons.

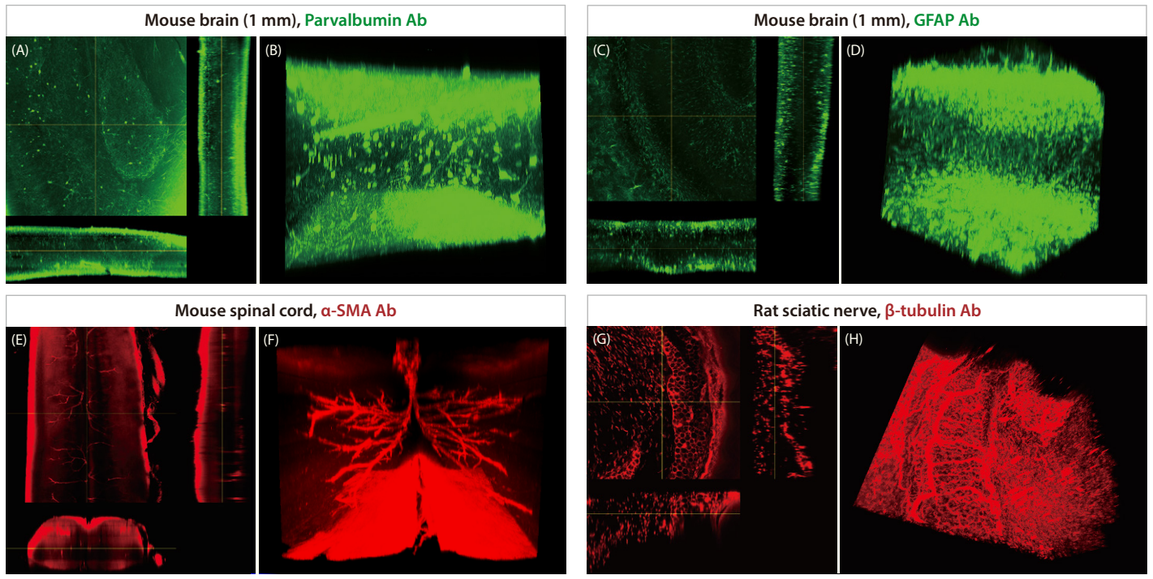


Figure 3. Immunolabeling various tissues with DeepLabel. DeepLabel facilitated the immunolabeling of various tissues with different antibodies. 1 mm brain slices were stained for Parvalbumin (A, B) and GFAP (C, D). Spinal cords were stained for α-SMA (E, F). Rat sciatic nerves were stained for β-tubulin (G, H).

Conclusion

DeepLabel Antibody Staining Kit:

- Facilitates the rapid and efficient penetration of macromolecular probes into thick, protein-dense tissues for site-specific binding.
- Reduces the time required for antibody incubation.
- Allows for vibrant fluorescence imaging at subcellular resolution.
- Has a simple protocol with ready-to-use reagents that are compatible with virtually all antibodies and all cleared tissues.

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