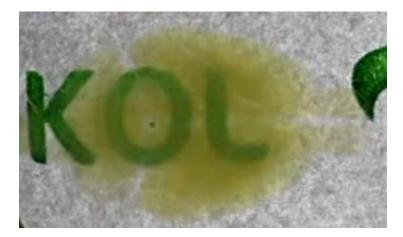
## **Protocol for Reversing Clearing of Tissue**

One of the main advantages of the Visikol® HISTO™ approach is that it is non-destructive and reversible, allowing traditional 2D histology to be conducted after 3D imaging. Because of the reversible nature of this approach, it can drop into the any bio-imaging process without disrupting the other assays and/or histological processing in traditional workflows. The process for reversing cleared is rapid and easy as described below:

- 1. Cleared tissue should be placed directly into a large volume of ethanol (absolute, or histological grade) or methanol. Volume of alcohol should be at least 10-20x tissue volume. Leave tissue at room temperature until opacity has been restored.
- 2. Larger and more vascular tissues (e.g. whole kidney) may require 2-3 washes of alcohol over the course of several hours.
- 3. After reversal, samples can be processed directly for paraffin embedding histological preparations.



Adult rat brain that has been rendered transparent with Visikol HISTO approach.



Adult rat brain after it has been reversed using ethanol gradient.