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We use this protocol and it's working

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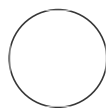
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## Immunohistochemistry data processing

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### ABSTRACT

This protocol has been used for high throughput quantification of immunohistochemistry data of mouse brain sections

### MATERIALS

1. Zeiss AxioScan.Z1 slide scanning microscope system (Carl Zeiss Inc., Thornwood, NY, USA) with a Plan-Apochromat 20x/0.8 objective lens.
2. Hamamatsu Orca flash 4.0 camera
3. The Zeiss ZEN blue 2.3 software
4. MediaCybernetics' Image-Pro 11 software package (Rockville, MD, USA).

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- 1 Widefield fluorescent images are collected for GFP and DAPI fluorescent channels using a Zeiss AxioScan.Z1 slide scanning microscope system with a Plan-Apochromat 20x/0.8 objective lens.
- 2 All images are acquired using a Hamamatsu Orca flash 4.0 camera with an average tile count of 165 tiles per brain section.
- 3 The Zeiss ZEN blue 2.3 software package was used for collection and stitching of the 2-color (DAPI & GFP) tiled images.
- 4 Widefield fluorescent images are then post-processed using MediaCybernetics' Image-Pro 11 software package (Rockville, MD, USA).
- 5 Every stitched image is processed using a protocol modified for each antibody. Initially, the image is masked to solely include the tissue in areas of interest.
- 6 Threshold segmentation is used for each antibody staining to separate actual signal from background auto-fluorescence.
- 7 Smart Segmentation is used to separate GFP-expressing puncta or fibrils from DAPI-stained nuclei in tissue evaluated with each antibody.
- 8 The Count/Size function is designed to extract the percent of the tissue sample that stains positive for GFP.

