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We use this protocol and it's working

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Tae-Un Han[1]

[1]National Institute of Health

Tae-Un Han

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.