Nov 17, 2025

Quantification of human mDA/progenitor cells in grafted animal brain tissue

Forked from Stereological estimation of human TH+ cells in grafted animal brain tissue

DOI
https://dx.doi.org/10.17504/protocols.io.14egnrwopl5d/v1

Roberto Garcia Swinburn[1], Ernest Arenas[1]

[1]Karolinska Institute Stockholm

SOX6 mDA differentiation

Roberto Garcia Swinburn
Karolinska Institute Stockholm

DOI: https://dx.doi.org/10.17504/protocols.io.14egnrwopl5d/v1

Protocol Citation: Roberto Garcia Swinburn, Ernest Arenas 2025. Quantification of human mDA/progenitor cells in grafted animal brain tissue. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egnrwopl5d/v1

Protocol status: Working
We use this protocol and it's working

Created: September 16, 2025

Last Modified: November 17, 2025

Protocol Integer ID: 227399

Keywords: grafted animal brain tissue, th positive cells within grafted animal, animal brain tissue, grafted animal, progenitor cell, human nuclei, positive cell

Abstract

We used this Qupath-based quantification for the percentage of human nuclei and TH positive cells within grafted animals with several other markers of interest.

Protocol materials

- OCT (Optimal Cutting Temperature compound) **Sakura Finetek Catalog #**4583
- Sheep Tyrosine Hydroxylase **Pel-Freez Catalog #**P60101-0
- Anti-Nuclei Antibody clone 235-1 **Merck Millipore (EMD Millipore) Catalog #**MAB1281
- DAPI **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**10236276001

Imaging

1 Slice the fixed frozen brain in 5 series at 20 µm using a Cryostat at -20 °C . The brain can be embedded in OCT (Optimal Cutting Temperature compound) **Sakura Finetek Catalog #**4583 to stabilize better. Each slice could be either in cold PBS1X or a cryoprotective solution for storage.

We ended with 15-25 slices of striatum with grafts.

2 Stain slices against TH, human nuclei and another marker of interest for mDA.
We used Sheep Tyrosine Hydroxylase **Pel-Freez Catalog #**P60101-0 , Anti-Nuclei Antibody clone 235-1 **Merck Millipore (EMD Millipore) Catalog #**MAB1281 and DAPI **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**10236276001 for the TH and human nuclei. The remaining channel was used for a marker of interest

3 Mount slices on glass slides.

4 Image human cells at 20X magnification. TH cells, human nuclei and DAPI-stained nuclei should be discernible.
We used Zeiss LSM880 confocal microscopes

Analysis

5 Creat a New Project on Qupath.

Software	
QuPath	NAME
Pete Bankhead	DEVELOPER
http://qupath.github.io	SOURCE LINK

6 Load images onto QuPath project

7 Create a Full Image annotation.

8 Use the Cell Detection tool (Analyze>Cell Detection>Cell Detection) to detect Human Nuclei.
Adjust the detection channel to the human nuclei and threshold to optimize detection.

9 Use the Classify tool (Classify>Object Classification>Create composite classifier). Adjust channel filter to TH channel, and threshold for optimized classification.
Above threshold should be TH, below should display nothing.
We recommend activating Live Preview to optimize Threshold.

Save the classifier.

10 Repeat step 9 with another channel with associated marker

11 Create a composite classifier (Classify>Object Classification>Create composite classifier) with your relevant markers.

12 Once you have saved the Cell Detection parameters and the Classification, re-use on all images.

13 Export the Image measurements in CSV

14 Calculate the percentage of double positive cells