Nov 16, 2025

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

DOI
https://dx.doi.org/10.17504/protocols.io.ewov11zbkvr2/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.ewov11zbkvr2/v1

Protocol Citation: Roberto Garcia Swinburn, Ernest Arenas 2025. Stereological estimation of human TH+ cells in grafted animal brain tissue. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov11zbkvr2/v1

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

Created: September 16, 2025

Last Modified: November 16, 2025

Protocol Integer ID: 227393

Keywords: grafted animal brain tissue, th positive cells within grafted animal, animal brain tissue, grafted animal, total number of human nuclei, stereological estimation, modified stereological estimation, human nuclei, positive cell

Abstract

We used this modified stereological estimation to quantify the total number of human nuclei and TH positive cells within grafted animals.

Protocol materials

- OCT (Optimal Cutting Temperature compound) **Sakura Finetek Catalog #**4583
- Anti-Nuclei Antibody clone 235-1 **Merck Millipore (EMD Millipore) Catalog #**MAB1281
- Donkey Anti-mouse AF647 **Invitrogen - Thermo Fisher Catalog #**A31571
- DAPI **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**10236276001
- Sheep Tyrosine Hydroxylase **Pel-Freez Catalog #**P60101-0
- Donkey anti-Sheep IgG (H L) Cross-Adsorbed Secondary Antibody Alexa Fluor 488 **Thermo Fisher Scientific Catalog #**A-11015

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.
We used Sheep Tyrosine Hydroxylase **Pel-Freez Catalog #**P60101-0 and Donkey anti-Sheep IgG (H L) Cross-Adsorbed Secondary Antibody Alexa Fluor 488 **Thermo Fisher Scientific Catalog #**A-11015
,
Anti-Nuclei Antibody clone 235-1 **Merck Millipore (EMD Millipore) Catalog #**MAB1281
and Donkey Anti-mouse AF647 **Invitrogen - Thermo Fisher Catalog #**A31571
DAPI **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**10236276001

3 Mount slices on glass slides from most rostral to most caudal.
We mounted several series with TH-stained slices (with other markers as well).

4 Image whole graft at 10X magnification. TH cells, human nuclei and DAPI-stained nuclei should be discernible.
We used Zeiss Axio Scan.Z1 Slide Scanner for a High-throughput analysis, and several series were imaged

Analysis

5 Creat a New Project on Qupath.

Software

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

6 Load images onto QuPath project

7 Encircle the graft (detected by human nuclei) with either the Polygon tool or the Brush tool.

8 Use the Cell Detection tool (Analyze>Cell Detection>Cell Detection) to detect Human Nuclei.
Adjust the detection channel to the human nuclei (in our case, AF647) and threshold to optimize detection.

9 Use the Classify tool (Classify>Object Classification>Create single measurement classifier). Adjust channel filter to TH channel (in our case AF488), and threshold for optimized classification.
Above threshold should be TH, below should display nothing.
We recommend activating Live Preview to optimize Threshold.

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

11 Export the Image measurements in CSV

12 Calculate the density of TH cells with human nuclei by dividing TH-positive objects per volume (area of graft * 20 µm)

13 Estimate total number:
$$Average Density of TH cells in slide * Number of slices * Number of series$$