Fluorescence intensity analyses

Ayse Ulusoy[1], Sinead O'Sullivan[1], Michael Helwig[1], Angela Rollar[1], Shirley Lee[1], Michael Klinkenberg[1], Rita Pinto-Costa[1], Donato Di Monte[1]

[1]DZNE

Ayse Ulusoy

AUG 02, 2023

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.5jyl8jrmdg2w/v1

Protocol Citation: Ayse Ulusoy, Sinead O'Sullivan, Michael Helwig, Angela Rollar, Shirley Lee, Michael Klinkenberg, Rita Pinto-Costa, Donato Di Monte 2023. Fluorescence intensity analyses. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl8jrmdg2w/v1

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

ABSTRACT

Fluorescence intensity analyses

Created: Aug 25, 2022

Last Modified: Aug 02, 2023

PROTOCOL integer ID: 69176

Keywords: ASAPCRN

1 Collect confocal z-stack images from fluorescent-labeled tissue samples. Here we used DHE-treated mice tissue counter-stained with human alpha-synuclein or tissue co-immunolabeled with a human alpha-synuclein and SynO2 antibodies.

Ox-DHE fluorescent signal measurement

2 Create a 3D surface rendering model of h-alpha-synuclein–immunoreactive DMnX neurons using the Imaris software.

3 By applying a constant intensity threshold select ox-DHE puncta and filter through h-alpha-synuclein–immunoreactive neuronal surfaces. This will allow for specific detection of ox-DHE puncta within immunoreactive neurons

4 Quantify puncta on a per-cell basis using the quantification tools.

Syn-O2 fluorescence intensity measurement

5 Generate 2D images that from 5 µm -thick z-stack images using maximum intensity projection function of the Zen software (Carl Zeiss).

6 Select human alpha-synuclein labeled neurons by applying a 120µm^2 size exclusion filter

7 Measure Syn-O2 intensity within these cells using the measure tool.