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Live imaging of synaptic vesicle dynamics in human iPSC-derived Dopamine Neurons (DaNs) using CypHer5E and FFN206

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

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Keywords: live imaging of synaptic vesicle pool, live imaging of synaptic vesicle dynamic, quantification of synaptic vesicle neurotransmitter loading, synaptic vesicle neurotransmitter loading, synaptic vesicle, active synaptic vesicle, synaptic vesicle pool, synaptic vesicle dynamic, synaptotagmin1, dopamine neuron, derived dopamine neuron, antibody to the luminal domain, fluorescent vmat2 substrate, using cypher5e, cypher5e method, fluorescence, labelled antibody, live imaging, content imaging

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Abstract

This protocol can be used for live imaging of synaptic vesicle pools and processes using two different labelling methods, CypHer and FFN206, which allows for the quantification of spot intensity, size and number using Harmony High-Content Imaging and Analysis Software by Revvity for semi-automated imaging processing.

The CypHer5E method images active synaptic vesicles using a fluorescence-labelled antibody to the luminal domain of Synaptotagmin1, while the FFN206 method provides quantification of synaptic vesicle neurotransmitter loading by VMAT2 using a fluorescent VMAT2 substrate.

Materials

Reagents:

- Antibody Synaptotagmin1 Luminal Domain IgG fluorescence-labelled with CypHer5E (Synaptic systems, CAT# 105 311CpH) - https://www.sysy.com/product/105311CpH
- BrainPhys™ Imaging Optimized Medium (StemCell technologies, CAT#05796) - https://www.stemcell.com/products/brainphys-imaging-optimized-medium.html
- FFN206 (Abcam, CAT# ab144554)
- NeuroFluor™ NeuO (StemCell technologies, CAT# 01801) - https://www.stemcell.com/products/neurofluor-neuo.html
- NucBlue™ Live ReadyProbes™ (Thermofisher Scientific, CAT# R37605) - https://www.thermofisher.com/order/catalog/product/R37605

Equipment:

- Opera Phenix™ Plus high-content imaging system (Revvity; CAT# HH14001000) - https://www.revvity.com/gb-en/product/opera-phenix-plus-system-hh14001000

Software:

- Harmony High-Content Imaging and Analysis software (Revvity, CAT# HH17000019) - https://www.revvity.com/gb-en/product/harmony-5-2-office-revvity-hh17000019#product-overview

Safety warnings

Dispose of all materials as per local guidelines.

Ethics statement

The protocols.io team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Before start

Produce dopamine neurons following **Protocol: Differentiation of human Dopamine Neurons (DaNs) from induced pluripotent stem cells** and half-feed twice weekly until the time point of Day 70. Replate on final replating day (typically, day 20) to a concentration of 50 000 cells/ well of a full area 96 well plate.

Live Imaging using CypHer5E

1 Remove media from plate and incubate cells in fresh media with Antibody Synaptotagmin1 Luminal Fraction Fluorescence-labelled with CypHer5E diluted at 1:100 for 12 hours at 37°C and 5% CO2.

Note

This is ideal to be done overnight, starting the incubation at the end of the previous work day and imaging the start of the next.

2 Remove all media from plate and incubate cells in 1:1000 NeuroFluor™ NeuO and NucBlue™ Live ReadyProbes™ fluorescence probes as per manufacturer's instructions (see **Materials**).

2.1 Incubate at 37°C and 5% CO2 for 30 minutes.

3 Replace media with fresh pre-warmed imaging optimised media (without phenol red) and image immediately at 37°C and 5% CO2.

Note

BrainPhys™ Imaging Optimized Medium supplemented with B27 works particularly well for longer term imaging.

Live Imaging using FFN206

4 Remove all media from cells and incubate neurons with FFN206 for 1 hour at 37°C and 5% CO2 and 1:1000 NeuroFluor™ NeuO.

5 Prior to imaging, replace media with fresh pre-warmed imaging optimized media (without phenol red) and image immediately at 37°C and 5% CO2.

Spot Analysis using Harmony High-Content Imaging and Analysis Software

6 Build a spot analysis pipeline using Harmony High-Content Imaging and Analysis Software as per Manufacturer's instructions using only NeuroFluor™ NeuroFluor™ NeuO and NucBlue™ Live ReadyProbes™ (for FFN206) or NeuroFluor™ NeuO and NucBlue™ Live ReadyProbes™ area (CypHer5E™™).

Note

A nuclei stain is preferred if a sufficient one is available (NucRed Live 647 ReadyProbes is an option, for example, when using the blue channel).