Sep 21, 2022

Fluorescent false neurotransmitter (FFN) live-cell DAT imaging

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dx.doi.org/10.17504/protocols.io.36wgqj55xvk5/v1

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ABSTRACT

This protocol describes how to measure the rate of uptake of FFN102, a fluorescent DAT substrate inside midbrain dopaminergic neurons.

DOI

dx.doi.org/10.17504/protocols.io.36wgqj55xvk5/v1

PROTOCOL CITATION

gurvir.virdi 2022. Fluorescent false neurotransmitter (FFN) live-cell DAT imaging. **protocols.io** https://protocols.io/view/fluorescent-false-neurotransmitter-ffn-live-cell-d-cgxetxje

CREATED

Sep 21, 2022

LAST MODIFIED

Sep 21, 2022

PROTOCOL INTEGER ID

70342

1 To measure the presence and activity of the DAT, we utilised the commercially available fluorescent DAT and VMAT2 substrate FFN102 (Abcam, ab120866).

2 To measure the uptake of the FFN102 dye, a field of view was first found using the brightfield settings on a Zeiss LSM 880 confocal microscope.

3 Cells were washed 1x with HBSS at room temperature

4 The cells then had 10 µM of the FFN102 dye in HBSS added and a time-series with an exposure every 5 seconds using the 405 nm laser was started, to measure uptake of the dye into the cells.

Excited using the 405 nm laser line and emission between 405 nm to 470 nm.

5 As a control to confirm specificity, samples in a different well were pre-treated with 5 µM of the DAT inhibitor nomifensine (Sigma) for 10 minutes in HBSS.

Nomifensine was kept in cell solution also after FFN102 was added.

6 Once the dye had entered cells, the cells were depolarised by the addition of 50 mM KCl to observe FFN102 dynamics.

7 Rate of fluorescence intensity increase inside cells was measured across the time-series to give a readout for the rate of FFN102 uptake through the dopamine transporter.

Citation: gurvir.virdi Fluorescent false neurotransmitter (FFN) live-cell DAT imaging
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