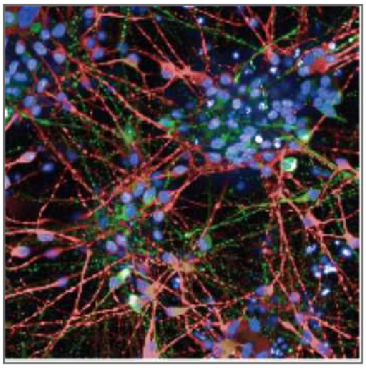

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Measuring dopamine release in human-derived iPSCs using High-Performance Liquid Chromatography (HPLC) coupled to Electrochemical Detection (ECD)

In 1 collection

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ABSTRACT

This protocol allows for the detection of evoked or tonic dopamine release from human iPSC-derived dopamine neurons using High-Performance Liquid Chromatography (HPLC) coupled to electrochemical detection.

GUIDELINES

This assay can be performed on any mature iPSC-derived dopamine neurons (DANs), but success has been particularly found on iPSC-DANs that have been grown in culture for 50-90 days following a modified Krik's protocol. Optimal density found to be plated at 500 000 / well in a 24 well cell culture dish. Collected samples can be stored long-term at -80^{o}C.

All steps are performed at Room Temperature (RT).

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

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MATERIALS

Reagents:

- 3,4-Dihydroxyphenylacetic Acid (Sigma-Aldrich, CAT# 11569-25MG)
- 5-Hydroxyindole-3-acetic Acid (Sigma Aldrich, CAT# H8876-5G)
- CaCl2
- Dopamine hydrochloride (Sigma-Aldrich, CAT# H8502-5G)
- Homovanilic acid (Sigma-Aldrich, CAT# 69673 - 25MG)
- KCl
- L-Norepinephrine hydrochloride (Sigma-Aldrich, CAT# 74480-100MG)
- MgCl2
- NaCl
- Phosphate-buffered saline (PBS), pH 7.4 (PBS) (Life Technologies, CAT# 10010056)
- Perchloric Acid (PCA)

Equipment:

- 4.6 x 150 mm Microsorb C18 reverse-phase column
- Decade II ECD
- Electrode (Antec Layden)

Making Ringers Buffer for evoked DA release:

Add each reagent to 500 mL dH2O

- 1.2 mM $CaCl_2$
- 148 mM NaCl
- 0.85 mM $MgCl_2$
- 40 mM KCl
- pH 7.4, 300 osm

Making Ringers Buffer for tonic DA release:

Add each reagent to 500 mL dH2O

- 1.2 mM $CaCl_2$
- 148 mM NaCl
- 0.85 mM $MgCl_2$
- 2.7 mM KCl
- pH 7.4, 300 osm

Making Mobile Phase Solution:

- 13% HPLC grade methanol
- 0.12 M Sodium phosphate monobasic dihydrate (NaH2PO4)
- 0.8 mM Ethylenediaminetetraacetic acid (EDTA)

- 0.5 mM 1-Octanesulphonic acid sodium salt (OSA)
- pH 4.6

Preparation

1 Add 1 µL of perchloric acid (PCA) to a labelled light-protected 1.5 mL Eppendorf tube for each sample.

2 Prepare Ringers Buffers for tonic or evoked release (see **Materials**). Store short-term at 4°C.

Dopamine (DA) Release Assay

3 Aspirate media from hiPSC-DANs plated as stated in guidelines.

4 Wash hiPSC-DANs one time with 100 µL phosphate buffered saline (PBS).

5 Aspirate PBS from hiPSC-DANs.

6 Add 100 µL of Ringer's Buffer for 5 minutes.

7 Collect Ringer's Buffer in pre-labelled tubes containing PCA.

8 Snap freeze on dry ice (optional).

Note

Samples can be stored at -80^{o}C long-term or immediately processed for HPLC.

HPLC-ECD

9 If snap-frozen and stored at -80^{o}C, thaw samples on ice.

10 Spin samples at 10 000 g for 10 minutes.

11 Run samples on HPLC column using a mobile phase running at 1 mL/min on a 4.6 x 150 mm Microsorb C18 reverse-phase column and Decade II ECD with a glassy carbon working electrode (Antec Layden) set at 0.7 V with respect to an Ag/AgCl reference electrode.

12 Calculate concentrate of dopamine released in sample compared to known standards.