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Quantification of glutamate release from human iPSC-derived Dopamine Neurons (DaNs)



Forked from Quantification of glutamate released from human induced pluripotent stem cells (iPSC) derived cortical neurons (CNs)

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We use this protocol and it's working

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Abstract

This protocol describes the procedure to quantify glutamate released from induced pluripotent stem cells derived dopamine neurons (iPSC-DANs) cultured in adherent monolayer following a modified Krik's protocol (Kriks, 2011) employing a progenitor expansions step as described in (Williamson and Madureira, 2023).

Materials

Reagents:

- [Glutamate Assay Kit](#) (Abcam, CAT# ab83389) - [Instructions for Use](#)
- [Hanks' Balanced Salt Solution \(HBSS++\)](#) (ThermoFisher, CAT# 14025092)
- [Potassium chloride \(KCl\)](#) (Sigma Aldrich, CAS# 7447-40-7)
- [Pierce™ BCA Protein Assay Kit](#) (ThermoFisher, CAT# 23225)

Equipment:

- 96-well microreader plate
- [PHERAstar® FSX microplate reader](#) (BMG LabTech)



Before start

Conditioned media used in this assay can come from any human cell type capable of producing and releasing glutamate. However, the protocol has been optimized for iPSC-derived dopamine neurons and plated at 1 million cells per well in a 12 well plate.

Prior to use, media must be warmed preferentially to 37°C as the cells are very temperature sensitive.

Plate cells in at least triplicate readouts for each cell line and/or condition.

Collection of Condition Media

1 Collection of conditioned media for tonic release:

- 1.1 Remove the cell culture media and add 200 μ l of pre-warmed HBSS++ (2.4 mM KCl) media to the cells.
- 1.2 Incubate the cells for 10 min before collecting the media.
- 1.3 Collect the media carefully and slowly making sure not to disturb the cells while adding and removing media.

2 Collection of conditioned media for evoked release:

- 2.1 Prepare 40 mM KCl HBSS++ solution by adding 29.82 mg KCl to 10ml of HBSS++. Filter sterilise prior to use.
- 2.2 Add 200 μ L of pre-warmed HBSS++ (40 mM KCl) media to the cells and incubate for 5 min. Collect the conditioned media.
- 2.3 Supernatants collected in both instances can be stored in -20°C following snap freezing until further use.

Measuring Glutamate in Conditioned Media

3 Preparation of standards:

- 3.1 Always prepare fresh set of standards on the day of use. The standards are unstable and needs to be used within 4 hours following preparation.
- 3.2 Prepare 1 mM of glutamate standard by diluting 5 μ L of 0.1 M glutamate with 495 μ L of the supplied Assay Buffer. Prepare a range of standards starting from 0 nmol to 10 nmol following the instruction booklet.

3.3 Prepare a range of lower concentrations of the standards in addition the concentrations mentioned in the manufacturer's instructions (**Step 3.2**) as often the glutamate released by the human neurons are in the lower concentration range (depending on the cell type and the seeded density).

4 **Assay Procedure:**

4.1 Bring all the materials and prepared reagents to room temperature before use.

4.2 Set up the reaction by adding 50 μ L of the standards and samples to wells of a 96-well microreader plate according to your plate map.

4.3 For calculating background for the samples, use 50 μ L of fresh HBSS++.

4.4 Add 100 μ L of reaction mix to each well and mix by pipetting up and down.

4.5 Incubate the plate at 37°C for 30 minutes protected from the light.

4.6 Read OD at 450 nm using a microplate reader for e.g. PHERAstar® microplate reader (BMG Labtech).

Analysis

5 Subtract blank values and average the data obtained in triplicates.

6 Plot a standard curve using the data obtained for the standards.

7 Calculate glutamate levels in nmol for each sample from the standard curve.

8 Normalise the glutamate release data for difference in plating density/cell number by dividing by the total protein values obtained by BCA.

