Feb 16, 2024

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

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.j8nlkoqdxv5r/v1

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.j8nlkoqdxv5r/v1

Protocol Citation: Humaira Noor, Richard Wade-Martins 2024. Quantification of glutamate released from human induced pluripotent stem cells (iPSC) derived cortical neurons (CNs). **protocols.io**
https://dx.doi.org/10.17504/protocols.io.j8nlkoqdxv5r/v1

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

Created: February 05, 2024

Last Modified: February 16, 2024

Protocol Integer ID: 94701

Keywords: Glutamate release, human neurons, tonic and evoked release, quantification of glutamate, induced pluripotent stem cell, cortical neuron, pluripotent stem cell, glutamate, neuron, cultured in adherent monolayer, ipsc, adherent monolayer

Funders Acknowledgements:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-020370

Abstract

This protocol describes the procedure to quantify glutamate released from induced pluripotent stem cells derived cortical neurons (iPSC-CNs) cultured in adherent monolayer.

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)
- **PierceTM BCA Protein Assay Kit** (ThermoFisher, CAT# 23225)

Equipment:

- 96-well microreader plate
- **PHERAstar® FSX microplate reader** (BMG LabTech)

Troubleshooting

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 optimised for iPSC-derived cortical neurons differentiated according to the following **Protocol: Differentiation of human cortical neurons (CNs) from induced pluripotent stem cells (iPSCs)**, and plated at 10,000 cells per well in half area 96-well plates and cultured in 100 µL of media.

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

Plate cells in a layout so that you have 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 60 µl 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 60 µL 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 µL of 0.1 M glutamate with 495 µL 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 µL 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 µL of fresh HBSS++.

4.4 Add 100 µL 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.