

Oct 05, 2022

High Performance Liquid Chromatography (HPLC) and sample preparation

DOI

dx.doi.org/10.17504/protocols.io.yxmvm2ko5g3p/v1

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DOI: dx.doi.org/10.17504/protocols.io.yxmvm2ko5g3p/v1

Protocol Citation: gurvir.virdi 2022. High Performance Liquid Chromatography (HPLC) and sample preparation. [protocols.io](https://dx.doi.org/10.17504/protocols.io.yxmvm2ko5g3p/v1)
<https://dx.doi.org/10.17504/protocols.io.yxmvm2ko5g3p/v1>

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Protocol status: Working

We use this protocol and it's working

Created: October 05, 2022

Last Modified: May 31, 2024

Protocol Integer ID: 70860

Keywords: ASAPCRN

Abstract

HPLC for metabolites in mDA neurons



Pre-sample prep

- 1 Prior to harvesting mDA neurons, all cells were incubated in phenol-free medium for 24 hours with and without the presence of [M] 80 micromolar (μM) L-Dopa (Sigma).

Extracellular metabolite sample prep

- 2 For extracellular metabolites, media after 24 hours was mixed 1:1 in [M] 0.8 Molarity (M) ice cold perchloric acid.
 - 2.1 Samples were incubated on ice for ⌚ 00:10:00 , followed by centrifugation at 🌀 12000 x g, 4°C, 00:05:00 . 15m
 - 2.2 The supernatant was removed and frozen in dry ice for HPLC analysis.

Intracellular metabolite sample prep


25m

- 3 For intracellular metabolites, after 24 hours, the cells were removed and pelleted by centrifugation at 🌀 1200 x g, 4°C, 00:05:00 . 5m
 - 3.1 The cells were washed once in PBS and lysed on ice using lysis buffer (10mM Tris (pH 7.4), 1mM EDTA, 320mM sucrose in HPLC grade water).
 - 3.2 Lysate was mixed with 1:1 in [M] 0.8 Molarity (M) ice cold perchloric acid and incubated on ice for ⌚ 00:10:00 . 10m
 - 3.3 The samples were centrifuged at 🌀 12000 x g, 4°C, 00:10:00 and the supernatant was harvested and frozen for HPLC analysis. 10m

HPLC

- 4 Quantification of neurometabolites (DOPAC, 3-OMD, 5-HIAA, HVA and dopamine) was carried out using reverse phase HPLC and an electrochemical detector



- 4.1 The mobile phase (flow rate 1.5ml/min) contained 16% methanol, 20mM sodium acetate trihydrate, 12.5mM citric acid monohydrate, 3.35mM 1-octanesulfonic acid, 0.1mM EDTA disodium and adjusted to pH 3.45 with 12 M hydrochloric acid (HCl).
- 4.2 The stationary phase was maintained at  27 °C .
- 4.3 The detector electrode was set at 450mV and screening electrode at 50mV.
- 4.4 50µl of sample was injected and calculated against a 500nM external standard solution of the 5 compounds of interest made in HPLC grade water acidified with 12M HCl.
- 4.5 Peak areas were quantified with EZChrom Elite™ chromatography data system software, version 3.1.7 (JASCO UK Ltd., Great Dunmow, UK).