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Striatal dopamine measurement through HPLC

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ABSTRACT

Protocol for striatal dopamine measurement in mouse brain

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MATERIALS TEXT

***PE Solution Recipe:* (250 mL*)*:**

Polished ddH2O-	249.06 mL
0.1mM EDTA (From 0.5 mM stock)-	50 uL
1uM DHBA (From 10mM stock)-	25 uL
50mM Phosphoric Acid (From 85% Fisher #A260 HPLC grade)-	855.3 uL

***Catecholamine Standard Prep*:**

Prepare 10 mM Stock in water or PE solution. (May be frozen as aliquots at –80C)

-Dopamine = 18.96 mg to 10 mL
(Dopamine hydrochloride, Sigma #H8502, MW 189.64)

-DOPAC = 16.82 mg to 10 mL
(3,4 Dihyroxyphenylacetic Acid, Acros #22560050, MW 168.15)

-HVA = 18.22 mg to 10 mL
(Homovanillic acid, MilliporeSigma #69673-25MG, MW 182.17)

-DHBA= 22.01 mg to 10 mL
(3,4 Dihydroxybenzlamine Hydrobromide, Sigma #858781, MW 220.06)
***(*INTERNAL STANDARD IN PE SOLUTION ONLY)*

Costar® Spin-X® Centrifuge Tube Filters, 0.22 µm Pore CA Membrane, Nonsterile - Cat#8161

1 Weigh the striatal tissue
Note - make sure to keep it frozen – can keep it on dry ice for the process

2 Add 20x PE buffer supplemented with an internal standard solution to tissue.
(eg. 10 mg sample tissue would receive 200 uL of PE solution)

3 Use a Teflon pestle to homogenize the tissue into the solution by crushing it against the walls of the tube.
Note - Do not sonicate as it may oxidize our analyte)

Note - Keep homogenized samples on ice until centrifugation

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Note - If using a hand homogenizer, note that the machine produces heat during prolonged use which can degrade the analytes. Allow time to cool between samples.

Alternatively, can use Dounce homogenizers

4 Centrifuge at **14000 rpm, 4°C, 00:15:00** 15m

5 Remove supernatant and transfer to Costar Spinx (#8161) 0.22 - MCA filter tube 10m
Centrifuge for **00:05:00** at **14.000 rpm, 4°C, 00:05:00** or until all liquid has passed through the filter.

Note - Filters have a max volume of 500uL, and may have to perform multiple spins/samples if the supernatant volume is >500ul.

6 The resulting solution can be run on HPLC or stored at **-80 °C** .

7 Running a standard - HPLC

Perform serial dilution of the stocks as follows to create the standard curve.

Level	[ng/mL]	uL stock	uL diluent
1	1000	1 from each 10 mM stock (DA/DOPAC/HVA)	997
2	600	429.8 from level 1	286.59
3	181.28	216.47 from level 2	286.59
4	54.77	216.47 from level 3	286.59
5	16.55	216.47 from level 4	286.59
6	5	216.47 from level 5	286.59

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8 Load at least 50 uL into each vial to run the standard curve in triplicate (on HPLC).

Run the samples

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