Monoamine oxidase activity in fish brain tissue

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Sep 09, 2020
1 Works for me
dx.doi.org/10.17504/protocols.io.bgu9jwz6

Fish behavior and physiology | Medicinal Plants Southeastern Pará Research Group

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ABSTRACT

The protocol describes a spectrofluorophotometric method for rapid determination of monoamine oxidase (MAO) activity in zebrafish brains. The protocol is based on the transformation of kynuramine hydrobromide into 4-hydroxyquinoline. Since zebrafish possess only one MAO isoform, inhibitors of other isoforms are not necessary.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Lima-Maximino M., Pyterson M. P., Carmo Silva R. X. do, Gomes G. C. V., Rocha S. P., Herculano A. M., Rosemberg D. B., Maximino C. (2020) Phasic and tonic serotonin modulate alarm reactions and post-exposure behavior in zebrafish. J. Neurochem. 153, 495–509. https://doi.org/10.1111/jnc.14978

DOI

dx.doi.org/10.17504/protocols.io.bgu9jwz6

PROTOCOL CITATION

Caio Maximino, Denis Broock Rosembeg 2020. Monoamine oxidase activity in fish brain tissue . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bgu9jwz6

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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KEYWORDS

Zebrafish, Monoamine oxidase

CREATED

May 26, 2020

LAST MODIFIED

Sep 09, 2020

PROTOCOL INTEGER ID

37505

MATERIALS

NAME	CATALOG #	VENDOR

NAME	CATALOG #	VENDOR
Trichloroacetic acid		P212121
NaOH		
Potassium Chloride		
Sucrose		
Disodium phosphate		
Monopotassium phosphate		
Centrifuge		
Standard Quartz cuvette		
Kynuramine hydrobromide	K3250	Sigma-aldrich
4-Hydroxyquinoline (4-Quinolinol)	H58005	Sigma – Aldrich
Spectrofluorophotometer		

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Kynuramine hydrobromide	K3250	Sigma-aldrich
Disodium phosphate		
Monopotassium phosphate		
sucrose		
Monopotassium phosphate		
Potassium Chloride		
NaOH		
Standard Quartz cuvette		
Spectrofluorophotometer		
4-Hydroxyquinoline (4-Quinolinol)	H58005	Sigma – Aldrich

EQUIPMENT

NAME	CATALOG #	VENDOR
Centrifuge	-	

SAFETY WARNINGS

Trichloroacetic acid (TCA) causes severe skin burns and eye damage, and may cause respiratory irritation. All solutions using TCA should be made using safety googles and nitrile rubber gloves, and vapors avoided by making preparations under a fume hood.
4-Quinolinol causes skin and eye irritation. All solutions using 4-Quinolinol should be made under a fume hood, with safety googles.

Reagent setup

1 Prepare **tissue lysis buffer**:
16.8 mM Na_2HPO_4 and 10.6 mM KH_2PO_4, pH **7.4** , isotonized with sucrose

Disodium phosphate

Monopotassium phosphate

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sucrose

2 Prepare **assay buffer**:
168 mM Na_2HPO_4 and 10.6 mM KH_2PO_4, pH **7.4** , isotonized with KCl

Disodium phosphate

Monopotassium phosphate

Potassium Chloride

Sample preparation

3

Euthanize fish in ice-cold water (<12 ºC), dissect brains in tissue lysis buffer. **Two brains should be pooled per to form one sample unit.**

4

Centrifuge samples on **0.5 mL** tissue lysis buffer, at 1.000 x *g* for 5 min.

Centrifuge -

5 Reserve supernatants, which will be used for assays, and maintain **On ice** throughout the experiment.

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Incubation 35m

6 5m

Mix protein samples (approximately 100 µg) with 460 µL of **assay buffer** and preincubated at **37 °C** for **00:05:00** (5 min).

7

Start reaction by adding [M]**110 Micromolar (µM)** kynuramine hydrobromide in a final volume of 700 µL.

Kynuramine hydrobromide
by Sigma-aldrich
Catalog #: K3250

8 30m

Incubate the final solution for **00:30:00** (30 min).

9 **Stop reaction** with 300 µL [M]**10 % volume** trichloroacetic acid.

Tricholoracetic acid is a strong acid that causes severe skin burns and eye damage, and may cause respiratory irritation. All solutions using TCA should be made using safety googles and nitrile rubber gloves, and vapors avoided by making preparations under a fume hood.

10

Centrifuge reaction products at 16.000 x *g* for **00:05:00** (5 min) and 800 µL of the supernatants mixed with 1 mL NaOH ([M]**1 Molarity (M)** .

NaOH

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Centrifuge -

Fluorescence measurement

11 Transfer centrifuged, NaOH-buffered reaction products to quartz cuvettes

Standard Quartz cuvette

12 Transfer cuvettes to a spectrofluorophotometer

Spectrofluorophotometer

13

Measure fluorescence using excitation at 315 nm and emission at 380 nm.

14

Concentration should be estimated with a standard curve of 4-hydroxyquinoline (4-Quinolinol)

4-Hydroxyquinoline (4-Quinolinol)
by Sigma – Aldrich
Catalog #: H58005

15

Calculate enzyme activity as nmol 4-OH quinoline/min/mg protein (determined by the Bradford or, preferably, Lowry assays).

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Lowry Protein Assay
by Irina Agarkova

PREVIEW RUN

15.1 Set up a standard curve of 0, 10, 25, 50, 100, 150 and 200 µg of BSA in 10-15 ml tubes, 1.0 mL volume per sample.

15.2 Set up tubes (10-15 mL) of the unknown protein sample(s) to determine their concentration, the final volume per sample is to be 1.0 mL.

May need to dilute the sample(s) so that some reactions are in the concentration range of the standard curve values.

15.3 Add 5.0 mL of reagent “C” to each tube, mix immediately.

15.4 Let the samples sit for 10 min at room temperature.
00:10:00

15.5 Add 0.5 mL of reagent “D” to each tube, mix immediately.

15.6 Let the samples sit for 30 min at room temperature.
00:30:00

15.7 Read absorbance at 600 nm. Record values, plot standard curve and determine sample concentration(s).

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