Sep 08, 2020

How to prepare zebrafish brain tissue samples for biochemical assays

Adrieli Sachett[1], Matheus Gallas-Lopes[1], Radharani Benvenutti [1], Greicy M M Conterato[2], Ana P Herrmann[1], Angelo Piato[1]

[1]Universidade Federal do Rio Grande do Sul; [2]Universidade Federal de Santa Catarina

1 *Works for me* dx.doi.org/10.17504/protocols.io.bjkdkks6

Angelo Piato

ABSTRACT

Zebrafish are increasingly used as a model animal in neuroscience research. Here we describe our protocol to collect and process zebrafish brains so they are well presearved and viable for biochemical assays.

DOI

dx.doi.org/10.17504/protocols.io.bjkdkks6

PROTOCOL CITATION

Adrieli Sachett, Matheus Gallas-Lopes, Radharani Benvenutti , Greicy M M Conterato, Ana P Herrmann, Angelo Piato 2020. How to prepare zebrafish brain tissue samples for biochemical assays. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bjkdkks6

KEYWORDS

Zebrafish, Brain tissue, Biochemical assays, Sample processing

CREATED

Aug 11, 2020

LAST MODIFIED

Sep 08, 2020

OWNERSHIP HISTORY

Aug 11, 2020 Matheus Gallas-Lopes Universidade Federal do Rio Grande do Sul

Sep 08, 2020 Angelo Piato

PROTOCOL INTEGER ID

40293

PARENT PROTOCOLS

Cited in

Protein quantification protocol optimized for zebrafish brain tissue (Bradford method)
Protein quantification protocol optimized for zebrafish brain tissue (Bradford method)
Quantification of nonprotein sulfhydryl groups (NPSH) optimized for zebrafish brain tissue
Quantification of nonprotein sulfhydryl groups (NPSH) optimized for zebrafish brain tissue
Quantification of thiobarbituric acid reactive species (TBARS) optimized for zebrafish brain tissue

Quantification of thiobarbituric acid reactive species (TBARS) optimized for zebrafish brain tissue

GUIDELINES

This protocol is intended to standardize the collection and processing of zebrafish brain tissue samples. It can be adapted for other fish species. Tissue amounts are adjustable depending on each laboratory standard pool and the aim of the biochemical assay.

MATERIALS

NAME	CATALOG #	VENDOR
Gloves		
Eppendorf tubes 1.5 mL uncolored	022363204	Eppendorf Centrifuge
MiniVortexer	58816-121	VWR Scientific
Surgical mask		
Pestle with a conical end		
Micropipette (0.5 - 10 µL)		
Micropipette (100 - 1000 µL)		
pH meter		
Thermal box		
Centrifuge 5424 R	5404000022	Eppendorf

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Phosphate buffered saline powder, pH 7.4, for preparing 1 L solutions	P3813	Millipore Sigma

SAFETY WARNINGS

Use personal protective equipment (including lab coat, masks, and gloves) when manipulating chemical and biological samples. Read the Safety Data Sheets of the reagents.

BEFORE STARTING

This protocol was standardized at LAPCOM (Psychopharmacology and Behavior Laboratory at UFRGS) to assess biochemical parameters in zebrafish brain tissue.

Preparations to collect animal tissue

1 Before starting to collect animal tissue, it is important to prepare some settings in order to guarantee the appropriate preservation of the sample and, ultimately, the assessment of biochemical parameters;

1.1 Fill a thermal box with shaved ice;

1.2 Prepare the **1.5 mL** microtubes that will be used to store tissue samples with the correct information. Microtubes used in this step should have a conical bottom to ensure the homogenization of the tissue using a pestle with a conical end;

1.3 After the microtubes are correctly identified, they must be buried halfway in the ice and filled with **150 µL** of phosphate buffered saline solution pH **7.4** at **4 °C** ;

Citation: Adrieli Sachett, Matheus Gallas-Lopes, Radharani Benvenutti , Greicy M M Conterato, Ana P Herrmann, Angelo Piato (09/08/2020). How to prepare zebrafish brain tissue samples for biochemical assays. https://dx.doi.org/10.17504/protocols.io.bjkdkks6

Phosphate buffered saline powder, pH 7.4, for preparing 1 L solutions
by Millipore Sigma
Catalog #: P3813

Sample collection and processing

2

The following steps should be carried out following animal welfare and ethical guidelines;

2.1 Euthanize the animals by exposure to chilled water between **2 °C** and **4 °C** until loss of orientation and cessation of opercular movements;

2.2 Two minutes after the loss of orientation and cessation of opercular movements, use a scalpel to remove the cranium of the fish to collect brain tissue;

2.3 Place each brain in the respective microtube with the help of the scalpel, making sure the tissue is immersed in the solution and the microtube stays in the ice;

2.4 After finishing the sample collection, use a pestle with a conical end to homogenize the tissue in the solution. Move the pestle up and down making circular movements to grind the tissue between the microtube and the pestle. Homogenize the samples for **00:01:00** (performing the circular movements around 60 times). The same researcher should homogenize all of the samples for better standadization of the process;

2.5 If you are using a pool of two or more brains, add **150 µL** of chilled phosphate-buffered saline solution to the tube for each additional brain;

2.6 Use a vortexer to mix the samples for **00:00:10** ;

2.7 Centrifuge the samples **3000 x g, 4°C, 00:10:00** ;

2.8 Use a micropipette to collect the supernatant and transfer it to a new microtube properly identified. Be careful to avoid the precipitate;

2.9 Store your samples in a freezer at **-80 °C** .

Citation: Adrieli Sachett, Matheus Gallas-Lopes, Radharani Benvenutti , Greicy M M Conterato, Ana P Herrmann, Angelo Piato (09/08/2020). How to prepare zebrafish brain tissue samples for biochemical assays. https://dx.doi.org/10.17504/protocols.io.bjkdkks6