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

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ABSTRACT

Zebrafish are increasingly used as a model animal in neuroscience research. Here we describe our protocol to quantify thiobarbituric acid reactive species (TBARS) in zebrafish brain tissue. TBARS levels are indicative of lipid peroxidation.

GUIDELINES

This protocol is intended to standardize quantification of thiobarbituric acid reactive species in zebrafish brain tissue samples. It can be adapted for other fish species.

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We use this protocol and it's working

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40416

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MATERIALS

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✂ Gloves **Contributed by users**

✂ Incubator **Contributed by users**

✂ 96 well plate **Contributed by users**

✂ Eppendorf tubes 1.5 mL uncolored **Eppendorf Centrifuge Catalog #022363204**

✂ Compact Digital Dry Bath/ Block Heater, Compact Dry Bath S, 100-240V, US plug **Thermo Fisher Catalog #88871001**

✂ Surgical mask **Contributed by users**

✂ Micropipette (0.5 - 10 μ L) **Contributed by users**

✂ Micropipette (100 - 1000 μ L) **Contributed by users**

✂ Multichannel pipette (5 μ L; 30-300 μ L) **Contributed by users**

✂ Adhesive tape **Contributed by users**

✂ Synergy™ HTX Multi-Mode Microplate Reader **Biotek**

STEP MATERIALS

✂ Trichloroacetic acid (TCA) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T6399**

✂ Thiobarbituric acid (TBA) **J.T. Baker**

✂ Malondialdehyde tetrabutylammonium salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #36357**

✂ Ethanol **Merck Millipore (EMD Millipore) Catalog #100983**

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SAFETY WARNINGS

- ⚠ Use personal protective equipment (including lab coat, masks, and gloves) whenever manipulating chemical and biological samples. Make sure to read all Safety Data Sheets for the reagents.

BEFORE START INSTRUCTIONS

This protocol was standardized at LAPCOM (Psychopharmacology and Behavior Laboratory at UFRGS) to assess biochemical parameters in zebrafish brain tissue. Protocols you should read before proceeding with this method:

CITATION

Adrieli Sachett, Matheus Gallas-Lopes, Radharani Benvenuti, Greicy M M Conterato, Ana Herrmann, Angelo Piato. How to prepare zebrafish brain tissue samples for biochemical assays.

LINK

<https://protocols.io/view/how-to-prepare-zebrafish-brain-tissue-samples-for-bjkdkks6>

CITATION

Adrieli Sachett, Matheus Gallas-Lopes, Greicy M M Conterato, Radharani Benvenuti, Ana Herrmann, Angelo Piato. Optimized protein quantification protocol for zebrafish brain tissue (Bradford method).

LINK

<https://protocols.io/view/optimized-protein-quantification-protocol-for-zebr-bjnfkmbn>

Preparing the reagents

- 1 The first step is to prepare the reagents to be used in the quantification of thiobarbituric acid reactive species (TBARS) in the samples;
 - 1.1 **Trichloroacetic acid (TCA) 20% + Thiobarbituric acid (TBA) 0.5%:** this reagent should be prepared on the day of the biochemical assay, it should not be stored for later use;
 - 1.1.1 Weigh 2 g of TCA in a beaker of appropriate size;

Trichloroacetic acid (TCA) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T6399**
 - 1.1.2 Dissolve the TCA with 8 mL of ultrapure water;
 - 1.1.3 Weigh carefully 0.05 g of TBA in a piece of aluminum foil;

Thiobarbituric acid (TBA) **J.T. Baker**
 - 1.1.4 Add the TBA to the solution of water + TCA. Use a heating plate to help dissolve the

TBA in the solution;

1.1.5 Transfer your solution to a 10 mL volumetric flask;

1.1.6 Using ultrapure water, complete the solution's volume to reach 10 mL ;

1.2 Malondialdehyde (MDA): This reagent is volatile, be careful to maintain the storing flask closed;

Preparing a stock solution [M] 2000 nmol/mL :

1.2.1 Weigh carefully 0.00627 g of MDA in a piece of aluminum foil;

Malondialdehyde tetrabutylammonium salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #36357**

1.2.2 Transfer the MDA to a beaker of appropriate size;

1.2.3 Add, slowly, 10 mL of absolute ethanol to the beaker to dissolve the salt;

Ethanol **Merck Millipore (EMD Millipore) Catalog #100983**

1.2.4 Store the solution in an amber flask of appropriate size at 8 °C ;

1.2.5 Each time you proceed with this thiobarbituric acid reactive species quantification method, prepare a [M] 20 nmol/mL sample solution from your stock solution following the calculation below;

$$C1 \times V1 = C2 \times V2$$

$$[M] \text{ 2000 nmol/mL} \times V1 = [M] \text{ 20 nmol/mL} \times \text{ 5 mL}$$

$$V1 = \text{ 0.05 mL of the stock solution (50 μ L)}$$

1.2.6 Using a micropipette, collect 50 μ L of the stock solution and mix it to

4950 μ L of absolute ethanol;

Ethanol **Merck Millipore (EMD Millipore) Catalog #100983**

1.2.7 This diluted solution should be prepared on the day of the biochemical assay, it should not be stored for later use;

Incubation of the samples + standard curve

2

To optimize the reaction that forms the thiobarbituric acid reactive species, an incubation step is needed. Tissue sample collection and preparation are described elsewhere;

CITATION

Adrieli Sachett, Matheus Gallas-Lopes, Radharani Benvenuti, Greicy M M Conterato, Ana Herrmann, Angelo Piato. How to prepare zebrafish brain tissue samples for biochemical assays.

LINK

<https://protocols.io/view/how-to-prepare-zebrafish-brain-tissue-samples-for-bjkdks6>

- 2.1** Prepare 1.5 mL heat resistant microtubes, to be used to store the samples, with the correct information. The number of microtubes depends on the number of samples and points of the standard curve;
- 2.2** For each point of the standard curve, fill the plastic microtubes as described below. You should provide duplicates or triplicates of each point of the curve to make your quantification more precise. Using a micropipette fill the tubes in this order: MDA solution, ultrapure water, and TCA + TBA solution (mixing the solution with the pipette tip to homogenize the content);

Point of the curve	MDA (20 nmol/mL) (μL)	Ultrapure water (μL)	TCA 2% + TBA 0.05% solution (μL)
Control (0 nmol of MDA)	0	100	150
Point 1 (0.4 nmol of MDA)	20	80	150
Point 2 (0.8 nmol of MDA)	40	60	150
Point 3 (1.2 nmol of MDA)	60	40	150
Point 4 (1.6 nmol of MDA)	80	20	150
Point 5 (2.0 nmol of MDA)	100	0	150

The final volume in all microtubes should be 250 μL.

- 2.3** Before preparing your samples for incubation, you must calculate the sample volume that corresponds to 50 μg of proteins. This calculation is based on the Bradford method described elsewhere;

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2.3.1 To estimate the volume of the sample corresponding to $50\text{ }\mu\text{g}$ of proteins, divide the amount of protein needed ($50\text{ }\mu\text{g}$) by the total amount of proteins in the sample quantified by the Bradford method (example below);

Volume of the sample needed for the assay (μL) = $50\text{ }\mu\text{g}$ / total amount of proteins in the sample $\mu\text{g}/\mu\text{L}$

2.4 For each tissue sample, fill the plastic microtubes as described below. You should provide duplicates or triplicates of each sample to make your quantification more precise. Using a micropipette fill the tubes in this order: sample, ultrapure water, and TCA + TBA solution (mixing the solution with the pipette tip to homogenize the content). The water volume depends on the volume of the sample. All microtubes should have a final volume of $250\text{ }\mu\text{L}$, so water is used so that every solution reaches this volume (e.g. $50\text{ }\mu\text{L}$ of the sample + $150\text{ }\mu\text{L}$ of the TCA + TBA solution + $50\text{ }\mu\text{L}$ of water);

Microtubes	Sample (μL)	Ultrapure water (μL)	TCA 2% + TBA 0.05% solution (μL)
Control	0	100	150
Sample	Depends on the volume of the sample corresponding to $50\text{ }\mu\text{g}$ of proteins.	Depends on the volume of the sample. Volume needed for the final solution in the tube to reach $250\text{ }\mu\text{L}$.	150

2.5 All microtubes should be correctly closed and sealed with adhesive tape to avoid that the microtubes open during the incubation period;

2.6 Incubate all your samples at $100\text{ }^{\circ}\text{C}$ for $00:30:00$ using a dry bath;

Reading your samples

- 3 Prepare to read the absorbance of your samples in a microplate reader;

- 3.1 Use a conventional 96-well microplate to run your samples. Before start pipetting, each well of the microplate should be marked for sample identification.. Standard curve samples and tissue samples can be read on the same plate. Transfer 200 μL of the content of each microtube to it's corresponding well in the microplate;
- 3.2 Read the absorbance of the samples at 532 nm in a microplate reader;

Calculating data and determinig results

- 4 The calculations are based on the MDA curve (similar to the calculation of the protein curve described in other protocols);

CITATION

Adrieli Sachett, Matheus Gallas-Lopes, Greicy M M Conterato, Radharani Benvenutti, Ana Herrmann, Angelo Piato. Optimized protein quantification protocol for zebrafish brain tissue (Bradford method).

LINK

<https://protocols.io/view/optimized-protein-quantification-protocol-for-zebr-bjnfkmbn>

- 4.1 Calculate the correction factor for your MDA standard curve;

4.1.1 Correction factor (CF): Subtract the absorbance value of the control point of the curve from the mean absorbance of the point you are calculating. Divide the concentration of MDA of the well/point of the curve by the resulting value from the subtraction before;

$$\text{CF} = \frac{\text{[] MDA (nmol)}}{\text{Mean Abs} - \text{Control Abs}}$$

4.1.2 Mean correction factor (MCF): The mean correction factor is calculated by the arithmetic mean of the correction factors for each point of the curve;

$$\text{MCF} = \frac{\sum \text{Correction factors}}{\text{Count of correction factors}}$$

or

$$\text{MCF} = \frac{F_{C1} + F_{C2} + F_{C3} + F_{C4} + F_{C5}}{5(\text{number of factors})}$$

- 4.2 The amount of thiobarbituric acid reactive species in your samples is calculated by multiplying the corrected mean absorbance of your sample to the mean correction factor calculated above;

$$\text{TBARS} = (\text{Mean absorbance of the sample} - \text{Mean absorbance of the control sample}) \times \text{MCF}$$

- 4.3 Results should be expressed as nmol of TBARS/mg of protein. To do so, divide the result obtained above for the amount of thiobarbituric acid reactive species in your sample by the amount of protein that you used of your sample (50 µg or 0.05 mg in this protocol).

$$\text{nmol TBARS/mg protein} = \text{nmol TBARS on the sample} / 0.05 \text{ mg of proteins}$$