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Protein quantification protocol optimized for zebrafish brain tissue (Bradford method)

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

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ABSTRACT

Zebrafish are incresingly used as a model animal in neuroscience research. Here we describe a protocol to quantify the total amount of proteins in zebrafish brain tissue.

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PROTOCOL CITATION

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Aug 12, 2020 Matheus Gallas-Lopes Universidade Federal do Rio Grande do Sul

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PARENT PROTOCOLS

Cited in

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

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

GUIDELINES

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

MATERIALS

NAME	CATALOG #	VENDOR
Gloves		
96 well plate		
Surgical mask		
Micropipette (0.5 - 10 µL)		
Micropipette (100 - 1000 µL)		
13x045 mm needles		
Multichannel pipette (5 µL; 30-300 µL)		
Synergy™ HTX Multi-Mode Microplate Reader		Biotek

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Bovine albumin fraction V	1870	INLAB
ortho-Phosphoric acid 85%	1005732500	Sigma-aldrich
Brilliant Blue G	27815	Sigma-aldrich
Ethanol	100983	Merck Millipore
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. Protocols you should read before proceeding with this method:

Adrieli Sachett, Matheus Gallas-Lopes, Radharani Benvenutti, Greicy M M Conterato, Ana Herrmann, Angelo Piato. How to prepare zebrafish brain tissue samples for biochemical assays.
https://protocols.io/view/how-to-prepare-zebrafish-brain-tissue-samples-for-bjkdkks6

Preparing the reagents

1 The first step is to prepare the reagents to be used in protein quantification;

1.1 ⚠

Bradford reagent: Prepare and use this reagent under dim or no light, making sure all glassware used is covered in aluminum foil to avoid photodegradation of the reagent. Prepare and use this

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reagent at room temperature;

1.1.1 Weigh **0.05 g** of Coomassie Brilliant Blue;

Brilliant Blue G
by Sigma-aldrich
Catalog #: 27815
CAS Number: 6104-58-1

1.1.2 Dissolve completely the Coomassie Brilliant Blue in **25 mL** of absolute ethanol in a beaker of appropriate size;

Ethanol
by Merck Millipore
Catalog #: 100983

1.1.3 Add **50 mL** of 85% orthophosphoric acid to the solution;

ortho-Phosphoric acid 85%
by Sigma-aldrich
Catalog #: 1005732500
CAS Number: 7664-38-2

1.1.4 Transfer your solution to a **500 mL** volumetric flask;

1.1.5 Using ultrapure water, complete the solution's volume to reach **500 mL** ;

1.1.6 Store the solution in a glass flask covered in aluminum foil;

1.2 **Albumin solution:** The dilution of this solution depends on the concentration needed to build your standard curve (in this case **[M]1 mg/mL**);

Preparing a stock solution of [M]10 mg/mL :

1.2.1 Weigh **0.1 g** of bovine albumin;

Bovine albumin fraction V
by INLAB
Catalog #: 1870
CAS Number: 9048-46-8

1.2.2 Dissolve completely the albumin powder in **9 mL** of ultrapure water using a beaker of appropriate size;

1.2.3 Transfer your solution to a **10 mL** volumetric flask;

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

1.2.5 Stock this solution at **-20 °C** , in samples of **1.5 mL** or **2 mL** using plastic microtubes;

1.2.5 Unfreeze one of those **[M]10 mg/mL** samples and dilute it to the concentration needed (

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[M]1 mg/mL) following the calculation below:

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

$$[M]10\ mg/mL \times V1 = [M]1.0\ mg/mL \times 10\ mL$$

$$V1 = 1\ mL \text{ of the stock solution } (1000\ \mu L)$$

1.2.6 Using a micropipette, collect 1000 µL os the stock solution and transfer it to a 10 mL volumetric flask;

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

1.3 **Phosphate buffered saline solution (PBS):** You should also prepare a PBS solution (pH7.4) as you will need 10 µl of the solution at room temperature to determine the control point of the standard curve.

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

Microplate preparation and protein quantification

2

Use a conventional 96-well microplate to run your samples. Once again, protein quantification using Bradford reagent should be performed under dim or no light, making sure the microplate is carefully covered in aluminum foil to avoid photodegradation of the reagent. Also, this step should occur at room temperature;

2.1 Before start pipetting, each well of the microplate should be marked for sample identification. Absorbance should be read no later than 01:00:00 after pipetting tissue samples;

2.2 Use one of the microtubes with the bovine albumin solution to generate the standard curve to quantify the proteins in your tissue go to step #1.2 ;

2.3 To generate the standard curve, fill the wells of your microplate 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 wells in this order: Bradford reagent, Phosphate buffered saline solution (PBS), and bovine albumin solution (mixing the solution with the pipette tip to homogenize the content of each well). Air bubbles should be perforated with a needle to avoid bias in the analysis;

Well/Point of the curve	Bradford reagent	PBS	Bovine albumin (1 mg/mL)
Control	190 µL	10 µL	-
1	198 µL	-	2 µL
2	196 µL	-	4 µL
3	194 µL	-	6 µL
4	192 µL	-	8 µL
5	190 µL	-	10 µL

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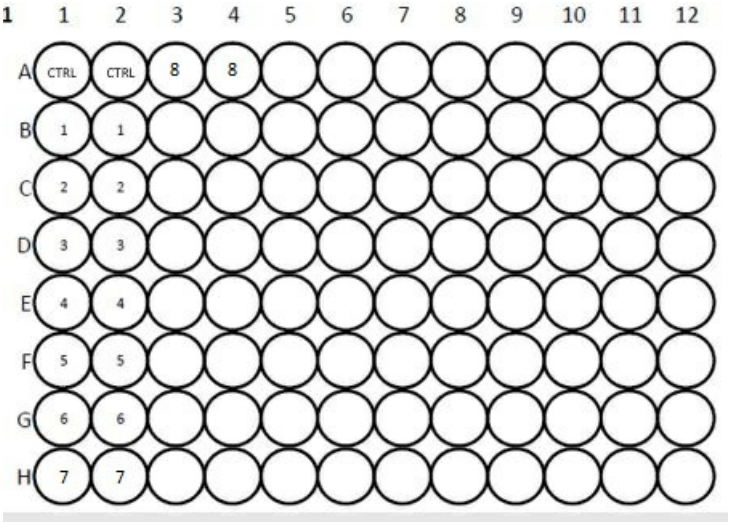
6	188 µL	-	12 µL
7	186 µL	-	14 µL
8	184 µL	-	16 µL



Example of the configuration of the plate to determine the standard curve. CTRL: control.

2.4 Read the absorbance of at **595 nm** in a microplate reader;

2.5 A demonstration of the expected results for the absorbances of the samples composing the curve is shown below. Calculate the mean absorbance to use in the further quantification of brain protein content;

Well/Point of the curve	Absorbance 1	Absorbance 2
Control	0.262	0.258
1	0.503	0.511
2	0.577	0.589
3	0.725	0.720
4	0.852	0.897
5	0.876	0.904

Use duplicates or triplicates of each point to assure that your quantification is precise.

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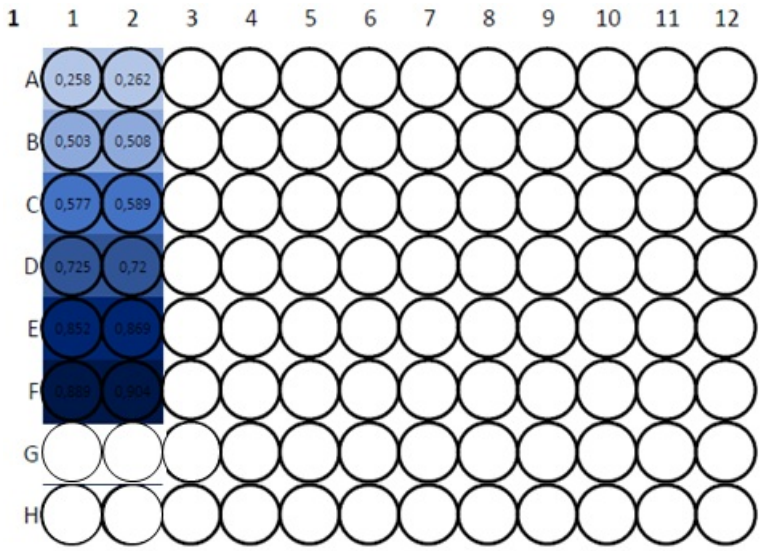


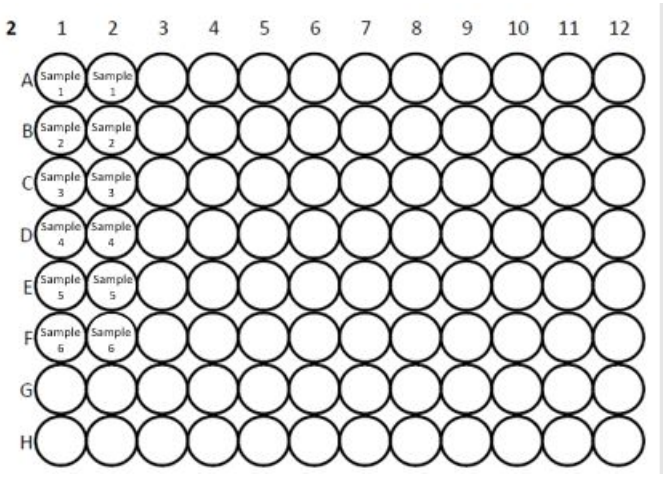
Example of the expected result in the plate. Different blue shades of blue are a result of the different quantities of protein present in each of the wells.

2.6 Following the determination of your standard curve, proceed to the quantification of your tissue samples. Using an adequate micropipette, fill the wells of your microplate as described below. You should provide triplicates or quadruplicates of each sample to make your quantification more precise. Using a micropipette fill the wells in this order: Firstly, fill the wells with the Bradford reagent followed by the tissue samples, mixing the solution with the pipette tip to homogenize the content of each well. Tissue sample collection and preparation are described elsewhere. The absorbance must be read in a maximum of **01:00:00** after pipetting the samples as stated above. Always use new tips for each sample and make sure that any researcher who handles the samples and plates is wearing a mask and gloves to avoid contamination. Air bubbles should be perforated with a needle to avoid bias in the analysis;

Adrieli Sachett, Matheus Gallas-Lopes, Radharani Benvenutti, Greicy M M Conterato, Ana Herrmann, Angelo Piato. How to prepare zebrafish brain tissue samples for biochemical assays.
https://protocols.io/view/how-to-prepare-zebrafish-brain-tissue-samples-for-bjkdkks6

Well/Point of the curve	Bradford reagent	Tissue sample
Sample 1	190 µL	10 µL
Sample 2	190 µL	10 µL
Sample 3	190 µL	10 µL
Sample (n)	190 µL	10 µL

Citation: Adrieli Sachett, Matheus Gallas-Lopes, Greicy M M Conterato, Radharani Benvenutti , Ana P Herrmann, Angelo Piato (09/08/2020). Protein quantification protocol optimized for zebrafish brain tissue (Bradford method). https://dx.doi.org/10.17504/protocols.io.bjnfkmbn



Example of the configuration of the plate to quantify the protein content of your sample.

2.7 Read the absorbance of the samples at **595 nm** in a microplate reader;

2.8 A demonstration of the expected results for the absorbances of the tissue samples is shown below;

Well/Point of the curve	Abs 1	Abs 2	Abs3	Abs 4	Me
Sample 1	*1.100*	*1.038*	*1.111*	*1.056*	-
Sample 2	*1.128*	*1.060*	*1.037*	*1.057*	-
Sample 3	0.787	0.784	0.737	***0.824***	0.7
Sample 4	***0.945***	0.841	0.876	0.851	0.8
Sample 5	*1.009*	0.969	*1.001*	0.950	0.9
Sample 6	0.727	*1.087*	*1.027*	***0.464***	-
Sample 7	*1.148*	*1.140*	*1.119*	*1.139*	-
Sample 8	*1.029*	*1.016*	0.956	*1.026*	-
Sample 9	*1.068*	*1.000*	0.991	0.991	0.9
Sample 10	***0.444***	***0.814***	0.885	0.891	0.8
Sample 11	*1.104*	*1.055*	*1.027*	*1.019*	-
Sample 12	*1.210*	*1.218*	*1.196*	*1.188*	-
Sample 13	*1.167*	***0.763***	0.839	0.829	0.8
Sample 14	***0.842***	0.726	0.740	0.750	0.7

What to look for when revising your data:

The corrected mean absorbance of the sample is calculated by: Mean absorbance - Mean absorbance of the control sample of the curve.

Any absorbance reading above 1 (in italic) must be disregarded as the quantification won't be precise. If all of your readings are above 1, follow step 3.

When taking readings in triplicates or quadruplicates, one or two of the absorbance values can be disregarded if they are significantly different from the others (in bold and italic) and the average can be

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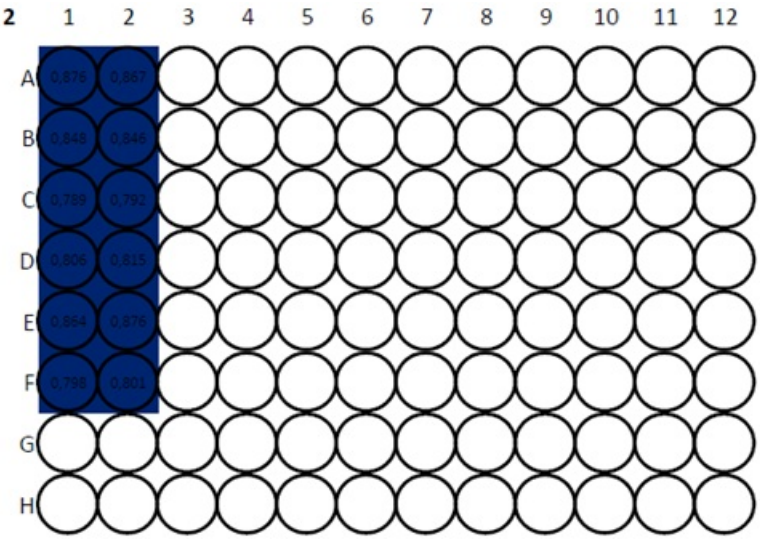
taken from the remaining values to ensure greater homogeneity of the data.



Example of the expected result in the plate. The blue color of the solution is directly related to the amount of protein present in each of the wells.

Protein quantification: optional steps

3

If the absorbance of your samples is above 1, the samples should be diluted in the portion of 1:2 (or even 1:3, 1:(n)) and go through a new reading phase as described above **go to step #2** , always remembering to multiply the final absorbance value obtained by the dilution factor (as shown in the calculations below).

Calculating data and determinig results

4

Follow the calculations below to get your results;

4.1 Calculate the correction factor for your bovine albumin 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 albumin of the well/point of the curve by the resulting value from the subtraction before;

$$CF = \frac{[\ ]\ Albumin}{Mean\ Abs - Control\ Abs}$$

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;

$$MCF = \sum Correction\ factors\ /\ Count\ of\ correction\ factors$$

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or

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

Using a solution of [M]**1 mg/mL** of bovine albumin and the expected results shown above:

Well/Point of the curve	Absorbance 1	Absorbance 2
Control	*0.262*	*0.258*
1	0.503	0.511
2	0.577	0.589
3	0.725	0.720
4	0.852	0.897
5	0.876	0.904

Correction factors:

Well/point of the curve: 1

Mean absorbance of the point - Control absorbance = 0.507 - 0.260 = **0.247**

[ ] Albumin: 1 mg – 1000 µL
x – 2 µL x = **2 µg**

CF_1 = 2 µg / 0.247 = **8.09717 µg/nm**

Well/point of the curve: 2

Mean absorbance of the point - Control absorbance = 0.583 - 0.260 = **0.323**

[ ] Albumin: 1 mg – 1000 µL
x – 4 µL x = **4 µg**

CF_1 = 4 µg / 0.323 = **12.38390 µg/nm**

Well/point of the curve: 3

Mean absorbance of the point - Control absorbance = 0.723 - 0.260 = **0.463**

[ ] Albumin: 1 mg – 1000 µL
x – 6 µL x = **6 µg**

CF_1 = 6 µg / 0.463 = **12.95896 µg/nm**

Well/point of the curve: 4

Mean absorbance of the point - Control absorbance = 0.875 - 0.260 = **0.615**

[ ] Albumin: 1 mg – 1000 µL
x – 8 µL x = **8 µg**

CF_1 = 8 µg / 0.615 = **13.00813 µg/nm**

Well/point of the curve: 5

Mean absorbance of the point - Control absorbance = 0.890 - 0.260 = **0.630**

[ ] Albumin: 1 mg – 1000 µL
x – 10 µL x = **10 µg**

CF_1 = 10 µg / 0.630 = **15.87302 µg/nm**

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Mean correction factor:

8.09717 + 12,38390 + 12,95896 + 13,00813 + 15,87302 = **62.32118 µg/nm**

MCF = 62,32118 / 5 = **12.464236 µg/nm**

4.2 Calculate the amount of protein of each of your tissue samples (the results should be expressed as µg/mL of proteins);

4.2.1 The amount of protein in your samples is calculated by multiplying the corrected mean absorbance of your sample to the mean corrected factor calculated above and dividing the result by the volume of the tissue sample used (in this case **10 µL**);

$$\text{Amount of protein} = \frac{\text{(Corrected mean absorbance x MCF)}}{10\mu L}$$

P.S. remember the corrected mean absorbance of the sample is calculated by subtracting the absorbance value of the control point of the standard curve from the mean absorbance of the sample that you are calculating.

4.2.2 If you are using diluted samples, remember to multiply the final absorbance value obtained by the dilution factor (DF).

$$\text{Amount of protein} = \frac{\text{(Corrected mean absorbance x MCF)} \times (DF)}{10\mu L}$$

P.S. the dilution factor depends on the proportion of the dilution applied:

DF (1:2) = 2
DF (1:3) = 3
DF (1:4) = 4

Well/Point of the curve	Abs 1	Abs 2	Abs3	Abs 4	Mea
Sample 1	*1.100*	*1.038*	*1.111*	*1.056*	-
Sample 2	*1.128*	*1.060*	*1.037*	*1.057*	-
Sample 3	0.787	0.784	0.737	***0.824***	0.76
Sample 4	***0.945***	0.841	0.876	0.851	0.85
Sample 5	*1.009*	0.969	*1.001*	0.950	0.96
Sample 6	0.727	*1.087*	*1.027*	***0.464***	-
Sample 7	*1.148*	*1.140*	*1.119*	*1.139*	-
Sample 8	*1.029*	*1.016*	0.956	*1.026*	-
Sample 9	*1.068*	*1.000*	0.991	0.991	0.99
Sample 10	***0.444***	***0.814***	0.885	0.891	0.88
Sample 11	*1.104*	*1.055*	*1.027*	*1.019*	-

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Sample 12	*1.210*	*1.218*	*1.196*	*1.188*	-
Sample 13	*1.167*	***0.763***	0.839	0.829	0.83
Sample 14	***0.842***	0.726	0.740	0.750	0.73

Calculating the amount of protein in the available samples above:

Sample 3:
(0.509 x 12.464236) / 10 = **0.634** Dilution factor (1:2): 0.634 x 2 = **1.269 µg/mL of proteins**

Sample 4:
(0.596 x 12.464236) / 10 = **0.743** Dilution factor (1:2): 0.743 x 2 = **1.486 µg/mL of proteins**

Sample 5:
(0.700 x 12.464236) / 10 = **0.872** Dilution factor (1:2): 0.634 x 2 = **1.745 µg/mL of proteins**

Sample 9:
(0.731 x 12.464236) / 10 = **0.911 µg/mL of proteins**

Sample 10:
(0.628 x 12.464236) / 10 = **0.783 µg/mL of proteins**

Sample 13:
(0.574 x 12.464236) / 10 = **0.715 µg/mL of proteins**

Sample 14:
(0.479 x 12.464236) / 10 = **0.597 µg/mL of proteins**

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