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



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Fish behavior and physi...

LAPCOM



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Protein quantification protocol optimized for zebrafish brain tissue (Bradford method). **protocols.io**

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We use this protocol and it's working

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Abstract

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

MATERIALS

-  Gloves
-  96 well plate
-  Surgical mask
-  Micropipette (0.5 - 10 μ L)
-  Micropipette (100 - 1000 μ L)
-  13×045 mm needles
-  Multichannel pipette (5 μ L; 30-300 μ L)
-  Synergy™ HTX Multi-Mode Microplate Reader **Biotek**

STEP MATERIALS

-  Bovine albumin fraction V **INLAB Catalog #1870**
-  ortho-Phosphoric acid 85% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1005732500**
-  Brilliant Blue G **Merck MilliporeSigma (Sigma-Aldrich) Catalog #27815**
-  Ethanol **Merck Millipore (EMD Millipore) Catalog #100983**
-  Phosphate buffered saline powder, pH 7.4, for preparing 1 L solutions **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P3813**

Protocol materials

- ✕ Phosphate buffered saline powder, pH 7.4, for preparing 1 L solutions **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P3813**
- ✕ Micropipette (0.5 - 10 µL)
- ✕ 13×045 mm needles
- ✕ Bovine albumin fraction V **INLAB Catalog #1870**
- ✕ ortho-Phosphoric acid 85% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1005732500**
- ✕ Gloves
- ✕ Brilliant Blue G **Merck MilliporeSigma (Sigma-Aldrich) Catalog #27815**
- ✕ Ethanol **Merck Millipore (EMD Millipore) Catalog #100983**
- ✕ Surgical mask
- ✕ Micropipette (100 - 1000 µL)
- ✕ Multichannel pipette (5 µL; 30-300 µL)
- ✕ Synergy™ HTX Multi-Mode Microplate Reader **Biotek**
- ✕ 96 well plate

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 start

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 (2026). How to prepare zebrafish brain tissue samples for biochemical assays.

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

LINK

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 reagent at room temperature;



1.1.1 Weigh 0.05 g of Coomassie Brilliant Blue;

Brilliant Blue G Merck MilliporeSigma (Sigma-Aldrich) Catalog #27815

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

Ethanol Merck Millipore (EMD Millipore) Catalog #100983

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

ortho-Phosphoric acid 85% Merck MilliporeSigma (Sigma-Aldrich) Catalog #1005732500

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 1 mg/mL);

Preparing a stock solution of 10 mg/mL :

1.2.1 Weigh 0.1 g of bovine albumin;

Bovine albumin fraction V INLAB Catalog #1870

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 10 mg/mL samples and dilute it to the concentration needed (1 mg/mL) following the calculation below:

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

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

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

1.2.6 Using a micropipette, collect 1000 μL of 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 (pH 7.4) as you will need 10 μL of the solution at room temperature to determine the control point of the standard curve.

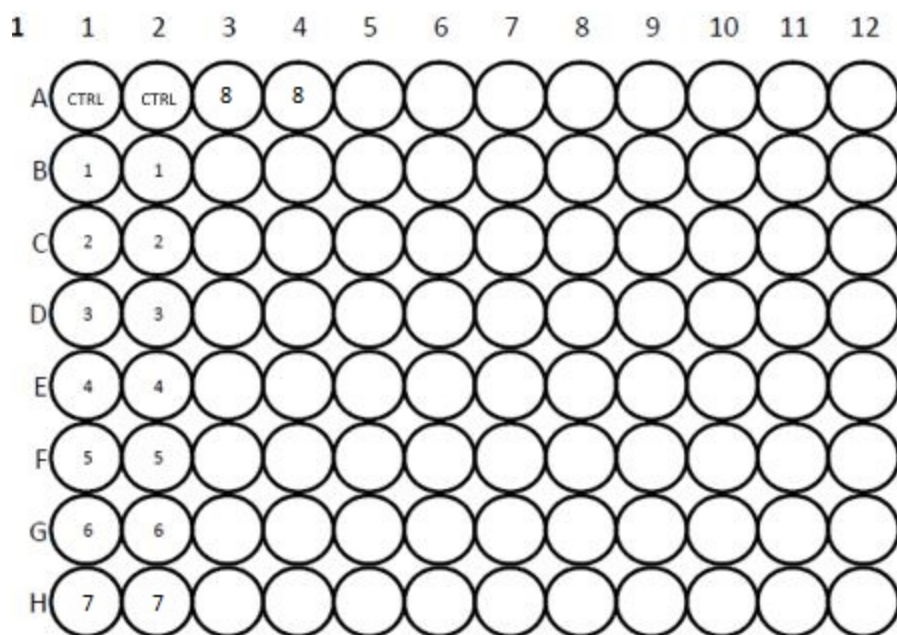
Phosphate buffered saline powder, pH 7.4, for preparing 1 L solutions **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P3813**

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
	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.

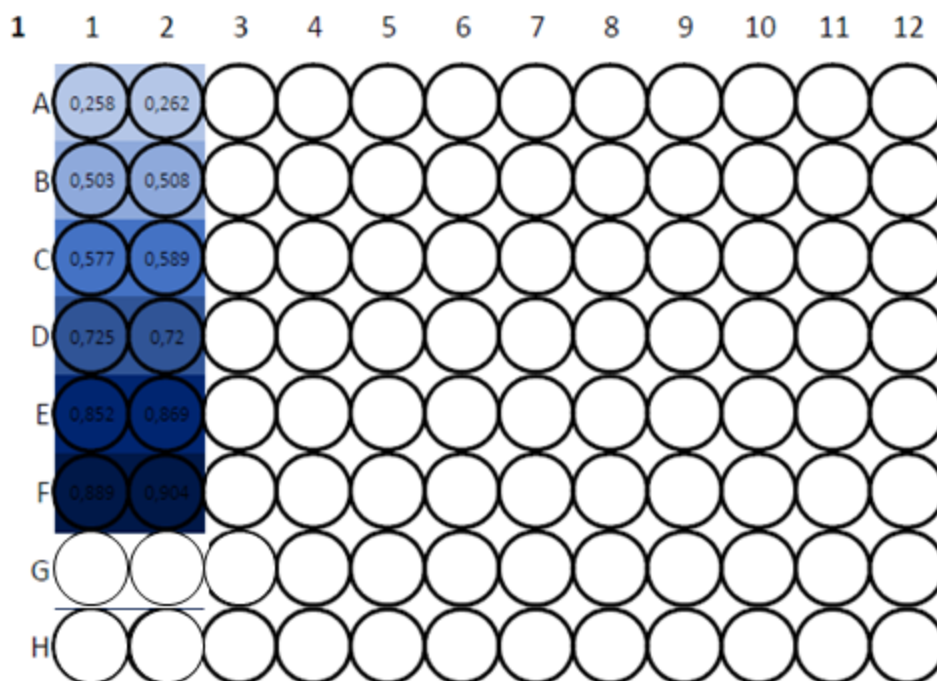
2.4 Read the absorbance of at 595 nm in a microplate reader;

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;

Expected result

	Well/Point of the curve	Absorbance 1	Absorbance 2	Mean
	Control	0.262	0.258	0.260
	1	0.503	0.511	0.507
	2	0.577	0.589	0.583
	3	0.725	0.720	0.723
	4	0.852	0.897	0.875
	5	0.876	0.904	0.890

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



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.

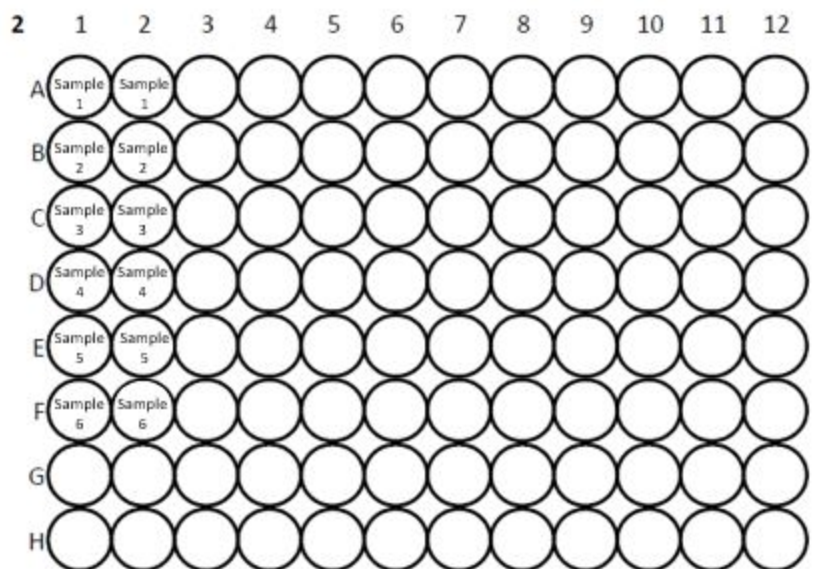
2.6 Following the determination of your standard curve, proceed to the quantification of your tissue samples. Using an adequate micropipette, fill the wells of your microplate as described below. You should provide triplicates or quadruplicates of each sample to make your quantification more precise. 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;

Citation

Adrieli Sachett, Matheus Gallas-Lopes, Radharani Benvenuti, Greicy M M Conterato, Ana Herrmann, Angelo Piato
(2026). How to prepare zebrafish brain tissue samples for biochemical assays.

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

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



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;

Expected result

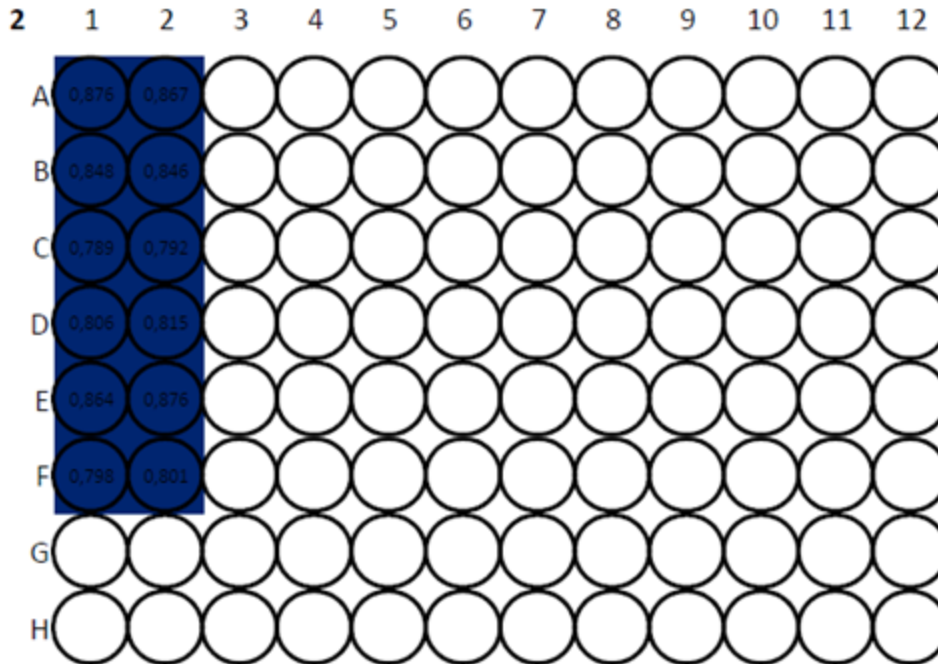
	Well/Point of the curve	Abs 1	Abs 2	Abs3	Abs 4	Mean	Corrected mean absorbance of the sample
	Sample 1	1.100	1.038	1.111	1.056	-	-
	Sample 2	1.128	1.060	1.037	1.057	-	-
	Sample 3	0.787	0.784	0.737	0.824	0.769	0.509
	Sample 4	0.945	0.841	0.876	0.851	0.856	0.596
	Sample 5	1.009	0.969	1.001	0.950	0.960	0.700
	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	0.991	0.731
	Sample 10	0.444	0.814	0.885	0.891	0.888	0.628
	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	0.834	0.574
	Sample 14	0.842	0.726	0.740	0.750	0.739	0.479

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 *italics*) 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 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 determininig 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;

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

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

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

Expected result

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	Mean
	Control	0.262	0.258	0.260
	1	0.503	0.511	0.507
	2	0.577	0.589	0.583
	3	0.725	0.720	0.723
	4	0.852	0.897	0.875
	5	0.876	0.904	0.890

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**

CF₁ = 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**

CF₁ = 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**

CF₁ = 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**



$$\begin{array}{rcl} [] \text{ Albumin:} & 1 \text{ mg} - 1000 \mu\text{L} & \\ & x & - 8 \mu\text{L} \\ & & x = 8 \mu\text{g} \end{array}$$

$$CF_1 = 8 \mu\text{g} / 0.615 = \mathbf{13.00813 \mu\text{g/nm}}$$

Well/point of the curve: 5

$$\text{Mean absorbance of the point} - \text{Control absorbance} = 0.890 - 0.260 = \mathbf{0.630}$$

$$\begin{array}{rcl} [] \text{ Albumin:} & 1 \text{ mg} - 1000 \mu\text{L} & \\ & x & - 10 \mu\text{L} \\ & & x = 10 \mu\text{g} \end{array}$$

$$CF_1 = 10 \mu\text{g} / 0.630 = \mathbf{15.87302 \mu\text{g/nm}}$$

Mean correction factor:

$$8.09717 + 12,38390 + 12,95896 + 13,00813 + 15,87302 = \mathbf{62.32118 \mu\text{g/nm}}$$

$$\text{MCF} = 62,32118 / 5 = \mathbf{12.464236 \mu\text{g/nm}}$$

- 4.2** Calculate the amount of protein of each of your tissue samples (the results should be expressed as $\mu\text{g/mL}$ 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);

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

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).

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

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

Expected result

	Well/Point of the curve	Abs 1	Abs 2	Abs3	Abs 4	Mean	Corrected mean absorbance of the sample
	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	0.769	0.509
	Sample 4	0.945	0.841	0.876	0.851	0.856	0.596
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	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	0.991	0.731
	Sample 10	0.444	0.814	0.885	0.891	0.888	0.628
	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	0.834	0.574
	Sample 14	0.842	0.726	0.740	0.750	0.739	0.479

Calculating the amount of protein in the available samples above:

Sample 3:

$(0.509 \times 12.464236) / 10 = \mathbf{0.634}$ Dilution factor (1:2): $0.634 \times 2 = \mathbf{1.269 \mu g/mL}$ of proteins

Sample 4:



$(0.596 \times 12.464236) / 10 = \mathbf{0.743}$ Dilution factor (1:2): $0.743 \times 2 = \mathbf{1.486 \mu\text{g/mL}}$ of proteins

Sample 5:

$(0.700 \times 12.464236) / 10 = \mathbf{0.872}$ Dilution factor (1:2): $0.634 \times 2 = \mathbf{1.745 \mu\text{g/mL}}$ of proteins

Sample 9:

$(0.731 \times 12.464236) / 10 = \mathbf{0.911 \mu\text{g/mL}}$ of proteins

Sample 10:

$(0.628 \times 12.464236) / 10 = \mathbf{0.783 \mu\text{g/mL}}$ of proteins

Sample 13:

$(0.574 \times 12.464236) / 10 = \mathbf{0.715 \mu\text{g/mL}}$ of proteins

Sample 14:

$(0.479 \times 12.464236) / 10 = \mathbf{0.597 \mu\text{g/mL}}$ of proteins

Citations

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

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

Step 2.6

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

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