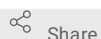# 🌐 Glutathione reductase (GR) activity assessment for zebrafish brain tissue

Jun 15, 2021

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<table>
<tr><td>1   Works for me</td><td>⤴ Share</td><td>dx.doi.org/10.17504/protocols.io.bsuuneww</td></tr>
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ABSTRACT

Zebrafish are being increasingly used as a model animal in neuroscience research. Here we describe a protocol to assess brain tissue activity of glutathione reductase (GR), an enzyme that catalyzes the reduction of the oxidized glutathione (GSSG) to the reduced form glutathione (GSH) which has a critical role in counterbalancing oxidative damage.

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GUIDELINES

This protocol is intended to standardize glutathione reductase activity assessment in zebrafish brain tissue samples. It can be adapted for other fish species.

MATERIALS TEXT

⊠Gloves **Contributed by users**   ⊠96 well plate **Contributed by users**

⊠1.5 mL Eppendorf tubes **Contributed by users**   ⊠Surgical mask **Contributed by users**

⊠Micropipette (0.5 - 10 µL) **Contributed by users**   ⊠Micropipette (100 - 1000 µL) **Contributed by users**

⊠pH meter **Contributed by users**   ⊠Synergy™ HTX Multi-Mode Microplate Reader **Contributed by users**

⊠Multichannel pipette (5 µL; 30- 300 µL) **Contributed by users**   ⊠Ultrapure water **Contributed by users**

⊠Monobasic potassium
phosphate **NUCLEAR Catalog #318312**   Step 1.1

⊠Potassium phosphate
dibasic **Neon Catalog #11361**   Step 1.1

⊠Ethylenediaminetetraacetic acid disodium salt dihydrate **Sigma
Aldrich Catalog #E5134-1KG**   Step 1.2

⊠L-Glutathione oxidized **Sigma Aldrich**   Step 1.3

⊠NADPH Tetrasodium salt **Sigma
Aldrich Catalog #10107824001**   Step 1.4

SAFETY WARNINGS

Use personal protective equipment (including lab coats, 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 , Greicy M M Conterato, Ana Herrmann, Angelo Piato. How to prepare zebrafish brain tissue samples for biochemical assays.
http://dx.doi.org/10.17504/protocols.io.bjkdkks6

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

Preparing the reagents

1   The first step is to prepare the reagents to be used in the assessment of glutathione reductase activity;

1.1   **Potassium phosphate buffer** [M]**154 Milimolar (mM)** :

1.1.1 Weigh ⬜**2.095 g** of monobasic potassium phosphate (KH_2PO_4) in a beaker of appropriate size;

⊗ Monobasic potassium phosphate **NUCLEAR Catalog #318312**

1.1.2 Dissolve the salt with ⬜**90 mL** of ultrapure water;

1.1.3 Transfer the solution to a ⬜**100 mL** volumetric flask;

1.1.4 Using ultrapure water, complete the solution's volume to reach ⬜**100 mL** ;

1.1.5 Weigh ⬜**2.682 g** of dibasic potassium phosphate (K_2HPO_4) in a beaker of appropriate size;

⊗ Potassium phosphate dibasic **Neon Catalog #11361**

1.1.6 Dissolve the salt with ⬜**90 mL** of ultrapure water;

1.1.7 Transfer the solution to a ⬜**100 mL** volumetric flask;

1.1.8 Mix both solutions slowly in a ⬜**500 mL** beaker following the steps below;

- Transfer ⬜**50 mL** of the monobasic potassium phosphate (KH_2PO_4) solution to the beaker;
- Use a pH sensor to evaluate your solution. Expected conditions: pH**7.0** ;

  If the pH of your solution is lower than 7 adjust the pH adding drops of the dibasic potassium phosphate (K_2HPO_4) solution;

  If the pH of your solution is above 7 adjust the pH adding drops of the monobasic potassium phosphate (KH_2PO_4) solution;

After adjusting the pH of this initial solution, proceed to add, slowly, drops of both buffer solutions (monobasic potassium phosphate and dibasic potassium phosphate). Use Pasteur pipettes to add the solutions. Mix your solutions using a pH sensor, making sure the mix of both buffers is always at pH**7.0** ;

1.2 EDTA (Ethylenediaminetetraacetic acid) buffer [M]**1.54 Milimolar (mM)** + Potassium phosphate buffer:

1.2.1 Weigh ⬜**0.057 g** of EDTA;

⊗ Ethylenediaminetetraacetic acid disodium salt dihydrate **Sigma Aldrich Catalog #E5134-1KG**

1.2.2 Dissolve completely the EDTA powder in ⬜**90 mL** of the potassium phosphate buffer solution prepared in the last step using a beaker of appropriate size;

1.2.3 Transfer your solution to a ⬜**100 mL** volumetric flask;

1.2.4 Using the potassium phosphate buffer solution prepared in the last step, complete the solution's volume to reach ⬜**100 mL** ;

1.2.5 Use a pH sensor to evaluate your solution. Expected conditions: pH**7.0** ;

1.2.6 Store this solution in an amber flask at 🌡 **8 °C** ;

1.3 Oxidized glutathione (GSSG) [M]**20 Milimolar (mM) :**

1.3.1 Weigh 🧪**0.0612 g** of GSSG;

⊠ L-Glutathione oxidized **Sigma Aldrich**

1.3.2 Dissolve completely the GSSG in 🧪**5 mL** of ultrapure water using a container of appropriate size;

1.3.3 Stock this solution at 🌡 **-20 °C** , in samples of 🧪**1 mL** using plastic microtubes;

1.4 NADPH (Dihydronicotinamide adenine dinucleotide phosphate tetrasodium salt) [M]**2 Milimolar (mM) :**

1.4.1 Weigh 🧪**0.016 g** of NADPH;

⊠ NADPH Tetrasodium salt **Sigma Aldrich Catalog #10107824001**

1.4.2 Dissolve completely the NADPH in 🧪**10 mL** of ultrapure water using a container of appropriate size;

1.4.3 Stock this solution at 🌡 **-20 °C** , in samples of 🧪**500 µL** using plastic microtubes;

Microplate preparation and absorbance reading	5m 5s

2 Use a conventional 96-well microplate to run your samples. Tissue sample collection and preparation are described elsewhere;

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

2.1 You must calculate the sample volume that corresponds to 🧪**30 µg** of proteins. This calculation is based on the Bradford method described elsewhere;

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

2.1.1 To estimate the volume of the sample corresponding to 🧪**30 µg** of proteins, divide the amount of protein needed (🧪**30 µg**) 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) = 30 µg / total amount of proteins in

2.2 Place and keep your Potassium phosphate buffer/EDTA ↻ **go to step #1.2** on a water bath at 🌡 **30 °C** throughout the analysis;

2.3 Set your microplate reader to the following configuration: Kinetic mode with agitation; 🔲 **340 nm** ; *5m 15s* Readings every ⏱ **00:00:15** for ⏱ **00:05:00** ; 🌡 **30 °C** ;

2.4 Using an adequate micropipette, fill the wells of your microplate as described below. You should provide duplicates or triplicates of each sample as stated above. Using a micropipette fill the wells in this order: Tissue sample, Potassium phosphate buffer/EDTA, and NADPH. Lastly, you must add the GSSG solution **as quickly as possible** using a multichannel pipette while being careful to homogenize the content of the wells. The Potassium phosphate buffer/EDTA volume depends on the volume of the sample. All wells should have a final volume of 🔲 **250 µL** , so the Potassium phosphate buffer/EDTA is used so that every solution reaches this volume (e.g., 🔲 **50 µL** of the sample + 🔲 **150 µL** of the Potassium phosphate buffer/EDTA + 🔲 **25 µL** of NADPH + 🔲 **25 µL** of GSSG);

	A	B	C	D	E
	Well	**Sample (µL)**	**Potassium phosphate buffer/EDTA 154 mM (µL)**	**NADPH 2 mM (µL)**	**GSSG 20 mM (µL)**
	Control	0	200	25	25
	Samples	Depends on the volume of the sample corresponding to 30 µg of proteins.	Depends on the volume of the sample. Volume needed for the final solution in the tube to reach 250 µL. (200 - sample volume)	25	25

2.5 Read the absorbance of the samples at the set conditions. You should see the absorbance of the *5m* sample wells decrease throughout the ⏱ **00:05:00** of measurement;

> Calculating data and determinig results   8m

3 Choose the best ⏱ **00:01:00** interval within the ⏱ **00:05:00** of sampling. To do so check for the interval where the *6m* decrease between absorbances is the greatest;

3.1 Calculate the mean absorbance of the wells containing the control solution and the wells containing your samples for each reading. Take into consideration whether you chose to use duplicates or triplicates of your samples.

3.2 Calculate the difference between the mean absorbance of the control samples within the ⏱ **00:01:00** *1m* interval that was chosen;

Δcontrol = (Mean absorbance at 0 s - Mean absorbance after 60 s)

3.3 Calculate the difference between the mean absorbance of each of your samples within the ⏲ **00:01:00** interval that was chosen;

1m

Δsample = (Mean absorbance at 0 s - Mean absorbance at 60 s)

3.4 Subtract the Δcontrol value from the Δsample value for each of the samples;

Absorbance of the sample (Δ min) = (Δsample - Δcontrol)

3.5 Determine GR activity:

GR activity = (Δ min x final volume (mL) x 1000000 x 1 mg/(6.22 x 1000 mL x amount of protein (mg))

GR activity = (Δ min x 0,25 mL x 1000000 x 1 mg/(6.22 x 1000 mL x 0,03 mg)

Simplifying: GR activity = (Δ min x 250000)/186.6

P.S.: 6.22 mM^{-1} cm^{-1} = molar extinction coefficients of NADPH

3.6 Final results are expressed as nmol NADPH/min/mg of protein.