Description

The GPX4 Fluorogenic Assay Kit is a fluorogenic assay designed to measure GPX4 (glutathione peroxidase 4) activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough purified recombinant GPX4, Glutathione, Cumene Hydroperoxide, NADPH, Glutathione Reductase, and GPX4 Assay Buffer for 100 reactions.

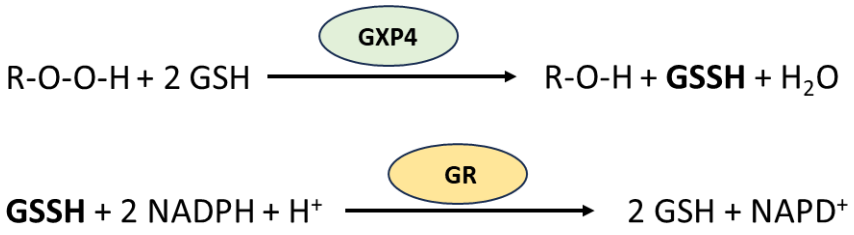

Figure 1: Mechanism of detection used in the GXP4 Assay Kit.

GXP4 activity is measured indirectly. GXP4 converts reduced glutathione (GSH) to oxidized glutathione (GSSG) by reducing hydroperoxide (ROOH). GSSG is then converted back to its reduced form by glutathione reductase (GR) in the presence of NAPDH, which is converted into $NAPD^+$. NADPH is intrinsically fluorescent, a property lost upon oxidation to $NADP^+$. The decrease in fluorescence (exc λ=340 nm; em λ=450 nm) observed is proportional to the amount of GSSH available for conversion by GR, and to GXP4 activity.

Background

GPX4 (glutathione peroxidase 4) belongs to the glutathione peroxidase family, which is composed of eight members (GPX1-8). GPX4 is unique in that it is the only monomeric member and can function in cell membranes. It is involved in fighting cellular oxidative stress by preventing membrane lipid peroxidation. Mammalian GPX4 contains a selenocysteine in its catalytic site crucial for reactions to occur and it thus a selenoprotein. GXP4 knock-out in mice results in embryonic lethality, indicating its unique role and limited number of pathways involved in cellular protection from lipid hydroperoxides. Dysfunction of GPX4 results in the accumulation of lipid peroxides and culminates in ferroptotic cell death. GPX4 has been found at high levels in cancer cells and contributes to drug-resistance against lapatinib, palbociclib and other anti-cancer drugs. The inhibition of GPX4 by (1S,3R)-RSL3 decreased the viability of drug-resistant cancer cells and reduced tumor size in mouse models. As the key regulator of ferroptosis and oncogene, GPX4 is thus a promising therapeutical target in cancer therapy.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
	GPX4*	25 µg	-80°C
	GPX4 Assay Buffer	5 ml	-20°C
	10 mM Glutathione	500 µl	-80°C
	5x Cumene Hydroperoxide (CHP)	100 µl	-80°C
	2.5 mM NADPH	400 µl	-80°C
	10x Glutathione Reductase	50 µl	-80°C
79685	Black 96-well plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- 50% Ethanol
- Microtiter-plate fluorimeter capable of excitation at λ=340 nm and detection of emitted light at λ= 450 nm
- Adjustable micropipettor and sterile tips

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Blank", "Positive Control" and "Test Inhibitor" conditions.
- We recommend using Mercury (II) Chloride as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1 x, 1 x and 10 x the IC_{50} value shown in the validation data below.
- We recommend maintaining the diluted proteins on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

1. Thaw **GPX4 Assay Buffer.**

2. Thaw **GPX4** on ice. Briefly spin the tube to recover its full content.

3. Dilute the protein (20 µl/well) to 12.5 ng/µl with GPX4 Assay Buffer.

4. Add 20 µl of diluted GPX4 to the wells designated “Positive Control” and "Test Inhibitor”.

5. Prepare the **Test Inhibitor** (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   5.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in GPX4 Assay Buffer, 10-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use GPX4 Assay Buffer (Diluent Solution).

   OR

   5.2 If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in GPX4 Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in GPX4 Assay Buffer to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in GPX4 Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

6. Add 5 µl of Test Inhibitor to each well labeled “Test Inhibitor”.

7. Add 5 µl of Diluent Solution to the “Positive Control" and “Blank” wells.

8. Add 20 µl of GPX4 Assay Buffer to the wells designated as "Blank”.

9. Preincubate the inhibitor with diluted **GPX4** for 30 minutes at Room Temperature RT.

10. Thaw **10 mM Glutathione**, **5x Cumene Hydroperoxide**, **2.5 mM NADPH** and **10x Glutathione Reductase**.

11. Dilute 10x Glutathione Reductase 10-fold in GPX4 Assay Buffer. This makes 1x Glutathione Reductase.

12. Prepare a **Master Mix** (20 µl/well): N wells x (5 µl of 1x Glutathione Reductase + 4 µl of 2.5 mM NADPH + 5 µl of 10 mM Glutathione + 6 µl of GPX4 Assay Buffer).

13. Add 20 µl of Master Mix to every well.

14. Incubate at RT for 5 minutes.

15. Dilute 5x Cumene Hydroperoxide 5-fold with **50% Ethanol.** This makes 1x Cumene Hydroperoxide.

16. Initiate the reaction by adding 5 µl of 1x Cumene Hydroperoxide to every well.

Component	Blank	Positive Control	Test Inhibitor
Diluted GPX4 (12.5 ng/µl)	-	20 µl	20 µl
Test Inhibitor	-	-	5 µl
GPX4 Assay Buffer	20 µl	-	-
Diluent Solution	5 µl	5 µl	-
	30 minutes at Room Temperature		
Master Mix	20 µl	20 µl	20 µl
	5 minutes at Room Temperature		
Diluted Cumene Hydroperoxide (1x)	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**

17. Incubate at RT for 10 minutes.

18. Read sample in a microtiter-plate fluorimeter capable of excitation at a wavelength of 340 nm and detection of emitted light of 450 nm.

19. The “Blank” value should be subtracted from all other values.

20. Data Analysis:

    a) Calculate the % of inhibition by

    % Inhibition = [(Blank Subtracted Positive Control – Blank Subtracted Test Inhibitor) / (Blank Subtracted Positive Control)] x 100.

    b) Calculate the % activity by:

    % Activity = [(Blank Subtracted Test Inhibitor) / (Blank Subtracted Positive Control)] x 100.

Example Results

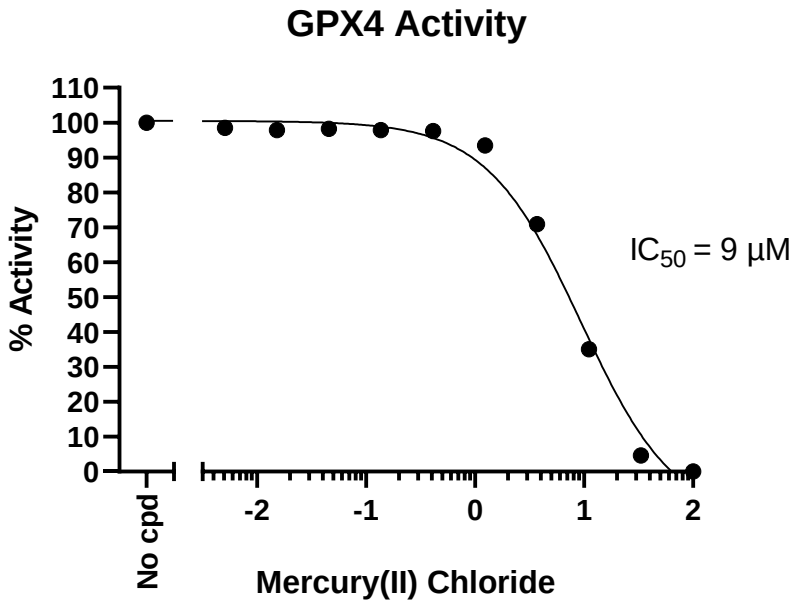

Figure 2: Inhibition of GPX4 activity by *Mercury (II) Chloride.* GPX4 activity was measured in the presence of increasing concentrations of Mercury (II) Chloride (Sigma #215465). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (activity in the absence of inhibitor, set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

Zhang X., *et al.*, 2020 *J Cell Physiol* 235 (4):3425-3437.
Lee J. and Roh J.-L., 2023 *Cancer Letters* 559: 216119.