TECHNICAL MANUAL

Glutathione Peroxidase 4 (GPX4) Activity Assay Kit

- **SKU CODE:** MAES0287
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add standards and samples
- Incubate and add reaction reagents
- Measure absorbance at 340 nm

2. Intended use

This kit can be used to measure GPX4 activity in animal tissue and cell samples.

3. Detection principle

Glutathione peroxidase 4 (GPX4) is a selenocysteine-containing glutathione peroxidase that reduces phospholipid hydroperoxides to phosphatidyl alcohols, thereby protecting cell membranes from oxidative damage.

GPX4 catalyzes the substrate, and the product consumes the reducing agent upon addition of enzyme reagents. The reducing agent has a maximum absorbance at 340 nm. The GPX4-specific enzyme activity can be calculated by measuring both non-specific enzyme activity and total enzyme activity after adding a GPX4 inhibitor to the system.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	25 mL x1 vial	50 mL x 1 vial	-20°C, 12 months
Reagent 2	Substrate	Powder x 1 vial	Powder x 2 vials	-20°C, 12 months, shading light
Reagent 3	Enzyme Reagent	0.25 mL x1 vial	0.5 mL x 1 vial	-20°C, 12 months, shading light
Reagent 4	Oxidant	0.15 mL x1 vial	0.3 mL x 1 vial	-20°C, 12 months, shading light
Reagent 5	Inhibitor	0.25 mL x1 vial	0.5 mL x 1 vial	-20°C, 12 months, shading light

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 6	Reducing Reagent	Powder x 1 vial	Powder x 2 vials	-20°C, 12 months, shading light
Reagent 7	Accelerant	4 mL x1 vial	8 mL x 1 vial	-20°C, 12 months, shading light
Reagent 8	Stabilizer	0.5 mL x1 vial	1 mL x 1 vial	-20°C, 12 months, shading light
	UV Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (340 nm), Incubator

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of extract working solution::** For each well, prepare 900 µL of extract working solution by mixing 10 µL of stabilizer with 890 µL of normal saline (0.9% NaCl). Keep extract working solution on ice during use. The extract working solution should be prepared fresh and used within 4 hours.
3. **Preparation of inhibitor working solution::** For each well, prepare 40 µL of inhibitor working solution by mixing 30 µL of buffer solution with 10 µL of inhibitor. Keep inhibitor working solution on ice during use. The inhibitor working solution should be prepared fresh and used within 1 day.
4. **Preparation of substrate working solution::** Dissolve one vial of substrate with 250 µL of double distilled water, mix well to dissolve completely. Store at -20°C for up to 2 days protected from light.
5. **Preparation of reducing working solution::** Dissolve one vial of reducing reagent with 500 µL of double distilled water, mix well to dissolve completely. Store at -20°C for up to 2 days protected from light.

6. **Preparation of oxidant working solution::** For each well, prepare 40 µL of oxidant working solution by mixing 38 µL of buffer solution with 2 µL of oxidant. Keep oxidant working solution on ice during use. The oxidant working solution should be prepared fresh and used within 2 hours.
7. **Preparation of reaction working solution::** Before testing, prepare sufficient reaction working solution according to the number of test wells. For example, prepare 200 µL of reaction working solution by mixing 108 µL of buffer solution, 2 µL of substrate working solution, 5 µL of enzyme reagent, 5 µL of reducing working solution, and 80 µL of accelerant. Keep reaction working solution on ice during use. The reaction working solution should be prepared fresh and used within 2 hours.

7. Sample preparation

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 50 mg tissue in 450 µL extract working solution with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant.

Cell sample:

1. Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 1×10^6 cells in 200 µL extract working solution with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10,000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant can be stored at 2-8°C for 1 day.

5. Meanwhile, determine the protein concentration of supernatant.

Note: The protein concentration of the sample should be greater than 0.2 g_{prot}/L, otherwise it will affect the accuracy of results.

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

10% Mouse liver tissue homogenate: 5-50

10% Mouse kidney tissue homogenate: 2-6

10% Mouse lung tissue homogenate: 2-5

2.05×10^6 293T cells: 1

1.9×10^6 4T1 cells: 1

2.03×10^6 Jurkat cells: 1

2×10^6 Molt-4 cells: 1

Note: The diluent is extract working solution. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. It is recommended to use fresh samples for detection.

2. As the sample reaction rate is fast, if A1 is not determined in time, it may lead to low or no value measured in the sample.

3. After adding oxidant working solution, measure the OD values of each well at 340 nm with microplate reader immediately within 15 seconds. It is better to measure no more than 10 wells at the same time.

4. The protein concentration of the sample should be greater than 0.2 g_prot/L, otherwise it will affect the accuracy of results.

9. Operating steps

1. Sample well: Add 20 µL of sample into sample well. Control well: Add 20 µL of sample into control well.

2. Add 40 µL of buffer solution into sample well. Add 40 µL of inhibitor working solution into control well.

3. Mix fully with microplate reader for 5 seconds, incubate at 37°C for 30 minutes.

4. Add 140 µL of reaction working solution into each well.

5. Add 40 µL of oxidant working solution into each well.

6. After adding oxidant working solution, measure the OD values of each well at 340 nm with microplate reader immediately within 15 seconds and record as A1. For tissue samples, incubate at room temperature (25°C) for 5 minutes, then measure the OD values of each well at 340 nm and record as A2. For cell samples, incubate at room temperature (25°C) for 15 minutes, then measure the OD values of each

well at 340 nm and record as A2. As the sample reaction rate is fast, if A1 is not determined in time, it may lead to low or no value measured in the sample.

10. Calculation

Tissue and cell samples:

Definition: The amount of GPX4 in 1 g protein per minute that catalyzes the substrate to produce 1 μmol substance at 25°C is defined as 1 unit.

GPX4 activity (U/g_{prot}) = (ΔA_sample - ΔA_control) ÷ (ε × d) × (V_{total} ÷ V_{sample}) ÷ T × f ÷ C_{pr}

[Note]

ΔA_sample: The change in OD value of sample well ($\Delta A = A_1 - A_2$)

ΔA_control: The change in OD value of control well ($\Delta A = A_1 - A_2$)

ε: The molar extinction coefficient of product at 340 nm, 6.22×10^{-3} L/μmol/cm

d: The optical path of cuvette, 0.6 cm

V_{total}: The total volume of reaction, 0.24 mL

V_{sample}: The volume of sample, 0.02 mL

T: The time of tissue sample reaction, 5 min; the time of cell sample reaction, 15 min

C_{pr}: The concentration of protein in sample, g_{prot}/L

f: Dilution factor of sample before test

Intra-assay Precision

Three mouse liver samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	6.80	16.20	32.50
%CV	3.1	1.4	1.2

Inter-assay Precision

Three mouse liver samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	6.80	16.20	32.50
%CV	3.4	2.2	0.4

Recovery

Three samples of high, medium, and low concentrations were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 98%.

Recovery

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	8.5	19	37
Observed Conc. (U/L)	8.5	18.3	36.1
Recovery rate (%)	100	96.3	97.7

Sensitivity

The analytical sensitivity of the assay is 3.22 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

11. Appendix II Example Analysis

Example analysis:

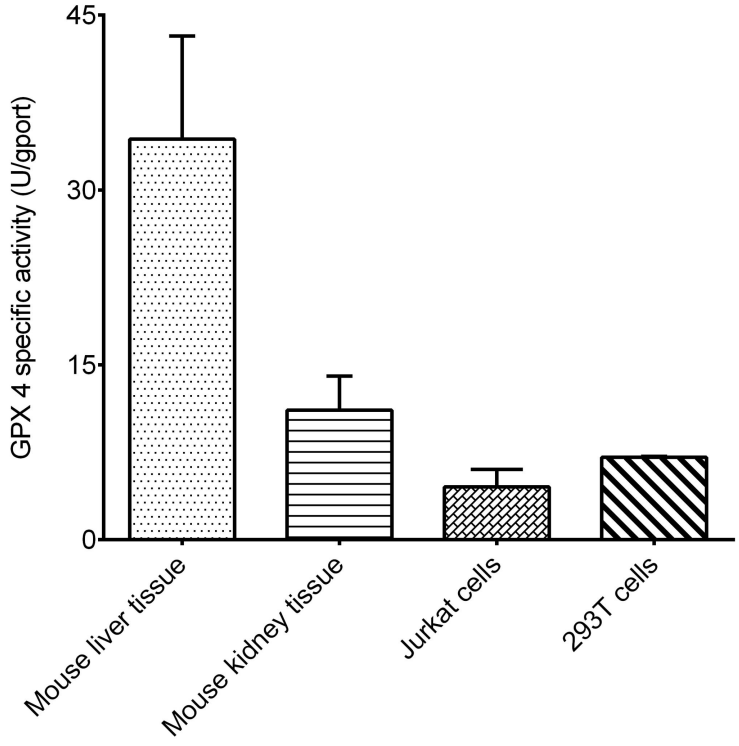
For 10% mouse liver tissue homogenate, dilute 10 times, take 20 μL of sample and carry out the assay according to the operation table. The results are as follows:

The OD value of the sample is 0.143, the OD value of the control is 0.100, the concentration of protein in sample is 6.177 g_{prot}/L and the calculation result is:

GPX4 activity (U/g_{prot}) = (0.143-0.100) ÷ (6.22 × 10^{-3} × 0.6) × (0.24 ÷ 0.02) ÷ 5 × 10 ÷ 6.177 = 44.76 U/g_{prot}

Detect 10% mouse liver tissue homogenate (protein concentration: 6.177 g_{prot}/L), 10% mouse kidney tissue homogenate (protein concentration: 5.492 g_{prot}/L), 2.7×10^6 Jurkat

cells homogenate (protein concentration: 0.639 g_{prot}/L), 2.03×10^6 293T cells homogenate (protein concentration: 1.635 g_{prot}/L), according to the protocol, the result is as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use

of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.