TECHNICAL MANUAL

Cell Glutathione Peroxidase (GPX) Activity Assay Kit

- **SKU CODE:** MAES0457
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add sample and reagents
- Measure absorbance
- Calculate GPX activity

2. Intended use

This kit can be used to measure glutathione peroxidase (GPX) activity in cell samples.

3. Detection principle

Glutathione peroxidase (GPX) plays an important role in protecting cells from free radical damage caused by hydrogen peroxide decomposition. The lipid components of cells are prone to react with free radicals, leading to lipid peroxidation. GPX uses glutathione to reduce peroxides to alcohols, thereby preventing cell damage.

GPX catalyzes the substrate, and the product consumes the reducing agent. The reducing agent has a maximum absorbance at 340 nm. The activity of GPX can be calculated by measuring the change in absorbance value at 340 nm.

4. Kit components & storage

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

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 6	Accelerant	4 mL × 1 vial	8 mL × 1 vial	-20°C, 12 months, protect from light
Reagent 7	Stabilizer	0.5 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months, protect from light
Reagent 8	Cell Lysis Buffer	7 mL × 1 vial	14 mL × 1 vial	-20°C, 12 months
	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

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate working solution::** Dissolve one vial of substrate with 250 µL of double distilled water and mix well to dissolve. Store at -20°C for 2 days protected from light.
3. **Preparation of oxidant working solution::** Before testing, prepare sufficient oxidant working solution according to the test wells. For example, prepare 100 µL of oxidant working solution (mix well 95 µL of buffer solution and 5 µL of oxidant). Keep oxidant working solution on ice during use. The oxidant working solution should be prepared fresh and used within 2 hours.
4. **Preparation of reductant working solution::** Dissolve one vial of reductant with 500 µL of double distilled water and mix well to dissolve. Store at -20°C for 2 days protected from light.
5. **Preparation of reaction working solution::** Before testing, prepare sufficient reaction working solution according to the test wells. For example, prepare 1285 µL of reaction working solution (mix well 825 µL of buffer solution, 10 µL of substrate working solution, 25 µL of enzyme reagent, 25 µL of reductant working solution and 400 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

1. **Cell sample preparation:** Cell (adherent or suspension) samples: 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 99 µL cell lysis buffer and 1 µL stabilizer. Incubate on ice for 10 minutes, mixing well every 5 minutes. 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 (MAES0177).
2. **Sample dilution:** The recommended dilution factor for different samples is as follows (for reference only): - 1.9×10^6 HL-60 cells: 1 - 1.4×10^6 293T cells: 1 - 2×10^6 CHO cells: 1 - 1.9×10^6 4T1 cells: 1 - 2.03×10^6 Jurkat cells: 1 Note: The diluent is cell lysis buffer. For the dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. The key points of the assay

If the A_2 of sample well is less than 0.2, the sample must be diluted and tested again.

9. Operating steps

1. Sample well: Add 20 µL of sample into sample well. Blank well: Add 20 µL of cell lysis buffer into blank well.
2. Add 180 µL of reaction working solution into each well.
3. Add 40 µL of oxidant working solution into each well.
4. Mix fully with microplate reader for 5 s. After adding oxidant working solution, measure the OD values of each well at 340 nm with microplate reader immediately and record as A_1. Incubate at room temperature for 10 min, then measure the OD values of each well at 340 nm and record as A_2. As the reaction rate is fast, if A_1 is not determined in time, it may lead to low or no value in the sample.

10. Calculation

Cell sample:

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

GPX activity (U/gprot) = (ΔA_sample - ΔA_blank) ÷ (ε × d) × (V_{total} ÷ V_{sample}) ÷ T × f ÷ C_{pr}

[Note]

ΔA_sample: The change in OD value of sample well (ΔA = A_1 - A_2)

ΔA_blank: The change in OD value of blank well (ΔA = A_1 - A_2)

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

d: Optical path, 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 reaction, 10 min.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three HL-60 cell 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/gprot)	98.53	116.90	129.05
%CV	3.10	1.20	1.50

Inter-assay Precision

Three HL-60 cell 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/gprot)	79.97	66.10	58.03
%CV	0.40	3.10	3.40

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with each concentration measured 6 times in parallel to get the average recovery rate of 100%.

Recovery

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/gprot)	102.29	116.90	131.51
Observed Conc. (U/gprot)	98.53	116.90	129.05
Recovery rate (%)	96.33	100.00	98.13

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.

12. Appendix II Example Analysis

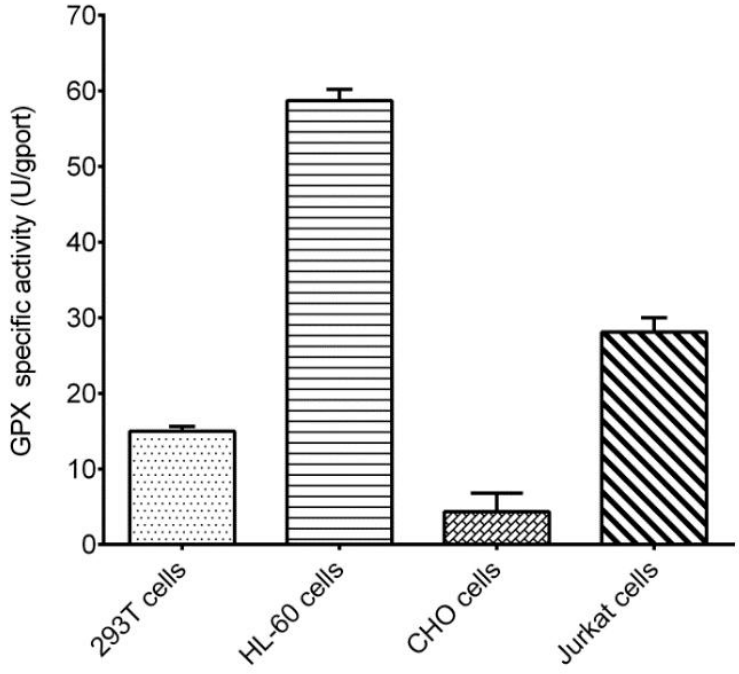
Example analysis:

For HL-60 cell, take 20 µL of supernatant and carry out the assay according to the operation table. The results are as follows:

The A_1 of the sample is 1.153, the A_2 of the sample is 0.851, $\Delta A_sample = A_1 - A_2 = 1.153 - 0.851 = 0.302$. The A_1 of the blank is 1.163, the A_2 of the blank is 1.157, $\Delta A_blank = A_1 - A_2 = 1.163 - 1.157 = 0.006$. The concentration of protein in sample is 1.698 gprot/L and the calculation result is:

GPX activity (U/gprot) = $(0.302 – 0.006) \div (6.22 \times 10^{-3} \times 0.6) \times (0.24 \div 0.02) \div 10 \div 1.698 = 56.05$ U/gprot

Detect 293T cell (the concentration of protein is 1.235 gprot/L), HL-60 cell (the concentration of protein is 1.698 gprot/L), CHO cell (the concentration of protein is 1.850 gprot/L) and Jurkat cell (the concentration of protein is 0.68 gprot/L) according to the protocol, the result is as follows:



13. 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 is not within 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.

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