Product Information

Glutathione Peroxidase Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2174-100 100 assays	Cap Color	Part Number
GPx Assay Buffer	25 ml	WM	K2174-C-1
NADPH (lyophilized)	1 vial	Blue	K2174-C-2
Glutathione Reductase	1 vial	Green	K2174-C-3
Glutathione (GSH; lyophilized)	1 vial	Brown	K2174-C-4
Cumene Hydroperoxide	1 vial	Yellow	K2174-C-5
GPx Positive Control (lyophilized)	1 vial	Red	K2174-C-6

II. Introduction:

Glutathione Peroxidase (GPx) family of enzyme protects cell from oxidative stress. GPx converts reduced glutathione (GSH) to oxidized glutathione (GSSG) while reducing lipid hydroperoxides to their corresponding alcohols or free hydrogen peroxide to water. Low levels of GPx have been linked to free radical related disease. In the Glutathione Peroxidase Activity Colorimetric Assay, GPx reduces Cumene Hydroperoxide as well as oxidizing GSH to GSSG. The generated GSSG is reduced to GSH with consumption of NADPH by GR. The decrease NADPH can be measured at 340 nm and is proportional to GPx activity. The assay can be used to detected glutathione dependent peroxidaes in tissue homogenates, plasma and cell lysates. The detection limit is ~ 0.5 mU/ml of GPx in samples.

III. Storage and Handling:

Store the kit at -20℃, protect from light. Warm the assay buffer to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

NADPH: Reconstitute with 0.5 ml dH_2O to get a 40 mM NADPH solution.

GR: Dilute with 0.22 ml Assay Buffer.

GSH: Reconstitute with 0.22 ml Assay Buffer.

Cumene Hydroperoxide: Dilute with 1.25 ml Assay Buffer. Mix well

GPx Positive Control: Reconstitute with 100 µl Assay Buffer.

All the solutions are stable for at least 1 week at 4℃ and 1 month at -20℃. Ensure that the assay buffer is at room temperature before use. Keep samples, GR mix solution and GPx Positive Control on ice during the assay.

V. Glutathione Reductase Activity Assay:

1. Sample Preparations:

Homogenize 0.1 g tissues, 10^6 cells, or 0.2 ml erythrocytes on ice in 0.2 ml cold assay buffer; Centrifuge at 10,000 x g for 15 min at 4℃; Collect the supernatant for assay and store on ice. Serum can be tested directly. Keep samples at -80℃ for storage. Add 2 - 50 µl of the samples into a 96-well plate; bring the volume to 50 µl with Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.

2. NADPH Standard Curve:

Dilute 25 µl of the 40 mM NADPH solution into 975 µl dH_2O to generate 1 mM NADPH standard. Add 0, 20, 40, 60, 80, 100 µl of the 1 mM NADPH Standard into 96-well plate in duplicate to generate 0, 20, 40, 60, 80,100 nmol/well standard. Bring the final volume to 100 µlwith Assay Buffer. Measure O.D. 340 nm to plot the NADPH Standard Curve.

3. Positive Control (optional) and Reagent Blank:

For Positive Control use 5 - 10 µl of the GPx Positive Control into the desired well(s) and adjust to 50 µl with Assay Buffer. Add 50 µl of Assay Buffer into a well (s) as a Reagent Control (RC).

4. Reaction Mix: For each well, prepare 40 µl Reaction Mix:

Assay Buffer	33 µl
40 mM NADPH solution	3 µl
GR solution	2 µl
GSH solution	2 µl

Add 40 µl of the Reaction Mix to each test samples, Positive Control (s) and RC(s) mix well, and incubate for 15 minutes to deplete all GSSG in your sample. Add 10 µl Cumene Hydroperoxide Solution to start GPx reaction. Mix well. Measure $OD_{340\ nm}$ at T1 to read A1, measure $OD_{340\ nm}$ again at T2 after incubating the reaction at 25°C for 5 min (or longer if the GPx activity is low) to read A2, protect from light. ΔA340 nm = [(Sample_A1 - Sample_A2) -(RC_A1 - RC_A2)]

Notes:

A. Measure the OD 340 nm before adding Cumene Hydroperoxide. Add more NADPH if the Sample OD at 340 nm is lower than 1.0 to ensure there is enough NADPH in the reaction system. 1 µl of 40 mM NADPH will give 0.5 OD at 340 nm.

B. If A1 reading is too low (< 0.7), it means either too much GPx or too much GSSG presence in the sample. You may need to dilute the samples, or remove GSSG from your sample using methods, such as dialyzing the sample or using spin filters to remove GSSG.

C. It is essential to read A1 and A2 in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose A1, A2, in the reaction linear range.

5. Calculation: Plot NADPH standard Curve. Apply the ΔA340nm to the NADPH standard curve to get NADPH amount B.

GPx Activity = ΔB/ [(T2−T1)× V] × Sample dilution = nmol/min/ml = mU/mL

Where: B is the NADPH amount that was decreased between T1 and T2 (in nmol).

T1 is the time of first reading (A1) (in min).

T2 is the time of second reading (A2) (in min).

V is the pretreated sample volume added into the reaction well (in ml).

Unit Definition: One unit is defined as the amount of enzyme that will cause the oxidation of 1.0 µmol of NADPH to $NADP^+$ under the assay kit condition per minute at 25℃.

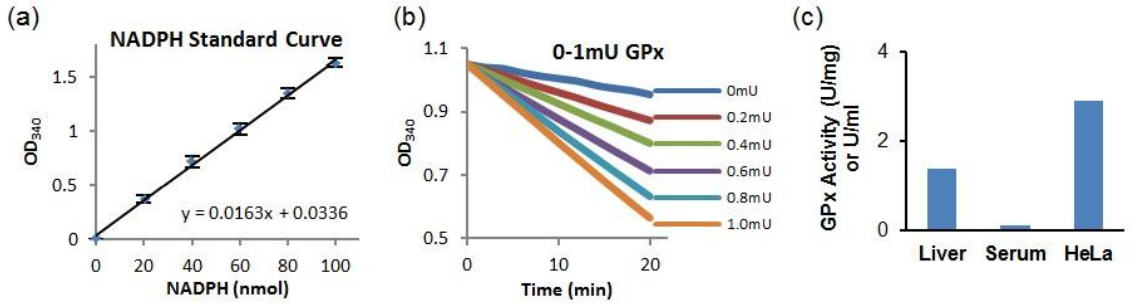

Figure: (a) NADPH Standard Curve. (b) Measurement of GPx activity using purified enzyme. (c) GPx Activity was measured using rat liver lysate (23 µg), human serum (1 µl) and HeLa cell lysate (16 µg). Assays were performed following the kit protocol.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the reaction mix • Air bubbles formed in well • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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