MA-0137

Glutathione Peroxidase Activity Assay

(100 wells, Colorimetric, OD 450 nm, Store at -20°C)

Background Information:

Glutathione peroxidase (GPx) is an enzyme that helps maintain redox balance and protects against oxidative stress. GPx catalyzes the reduction of H_2O_2 and organic hydroperoxides, using glutathione as a cosubstrate, preventing accumulation of ROS which cause damage to proteins, lipids, and DNA. GPx is widely distributed, particularly abundant in organs with high metabolic activity, (liver, kidney, lung, and red blood cells). Expression levels are modulated in response to environmental factors, including dietary selenium and oxidative stress levels. GPx deficiency is associated with pathologies, including neuro degenerative disease, cardiovascular disorders, cancer, and age-related macular degeneration. AkrivisBio's Glutathione Peroxidase Activity Assay is a simple, sensitive means of assessing GPx activity in a variety of samples with a sensitivity below 0.2 mU per well.

Assay Principle:

1 – In the presence of reduced glutathione, GPx reduces added cumene hydroperoxide, forming oxidized glutathione (GSSG) in the process.
2 – Glutathione reductase converts GSSG back to GSH, the reduced form of glutathione, with the conversion of NADPH to NADP.
3 – The decrease in NADPH is followed directly by monitoring the reaction at 340 nm.

Assay Components:

Assay Buffer	50 ml	NM	MA-0137A
NADPH	lyoph	Blue	MA-0137B
Glutathione Reductase	2 µl	Green	MA-0137C
Reduced Glutathione (GSH)	lyoph	Amber	MA-0137D
Cumene Hydroperoxide	2 µl	Yellow	MA-0137E
GPx Positive Control	lyoph	Red	MA-0137F

Storage and Handling:

Store the unopened kit at -20°C. Bring the assay buffer to RT before use. Centrifuge all small vials for a few seconds before opening.
Assay Buffer: Ready to use as supplied. Store at 4°C
NADPH: Reconstitute with 0.5 ml DI H_2O. Store at -20°C
Glutathione Reductase: Reconstitute with 220 µl Assay Buffer. Keep on ice while using. Store at -20°C
Reduced glutathione: Reconstitute with 220 µl Assay Buffer. Keep on ice while using. Store at -20°C.
Cumene Hydroperoxide: Reconstitute with 1.25 ml Assay Buffer. Store at 4°C.
Glutathione Peroxidase Positive Control: Reconstitute with 100 µl Assay Buffer. Keep on ice while using. Store at -20°C
Keep everything containing enzymes (samples, Glutathione Reductase, GPx Positive Control) on ice during use.

Assay Protocol:

1. Standard Curve: Transfer 25 µl of NADPH solution to 975 µl DI H_2O giving 1 mM NADPH. Add 0 – 20 – 40 – 60 – 80 – 100 µl of the 1 mM NADPH to a 96 well plate giving 0 – 20 – 40 – 60 – 80 – 100 nmoles. Adjust all wells to 100 µl with Assay Buffer. Measure O.D. at 340 nm to make the Standard Curve.

2. Sample Preparation: Homogenize tissue (10 mg), or cells ($10^{6)}$, or erythrocytes (100 µl pellet) in 100 µl Assay buffer, on ice. Centrifuge at 16,000 X g for 5 min at 4°C; Transfer the clear supernatant to a fresh tube and store on ice. Transfer serum directly to a well. Transfer 2 – 50 µl of sample to wells in a 96-well plate. Adjust all well volumes to 50 µl with Assay Buffer.
Note: NADPH is unstable and is easily degraded by a variety of enzymes. Samples not analyzed immediately should be stored at -80°C or colder. All sample readings must fall within the range of the standard curve. Any sample readings below about 0.3-0.4 OD are suspect of falling into the nonlinear regime and should be diluted and rerun.

3. Positive Control and Background Control: Transfer 5 µl of the GPx Positive Control to a well and adjust to 50 µl with Assay Buffer. Transfer 50 µl of Assay Buffer to a well as a background control to correct for any non-GPx loss of NADPH which may occur.

4. Prepare Reaction: Each reaction requires 40 µl of Pre-reaction mix. Prepare sufficient mix for the number of wells to be run:

	Pre-Reaction Mix:
Assay Buffer	33 µl
40 mM NADPH solution	3 µl
GR solution	2 µl
GSH solution	2 µl

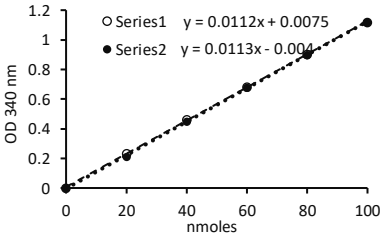
Add 40 µl of the Pre-reaction Mix to each test, Positive Control and background control well. Mix and preincubate 15 minutes at RT to deplete GSSG.

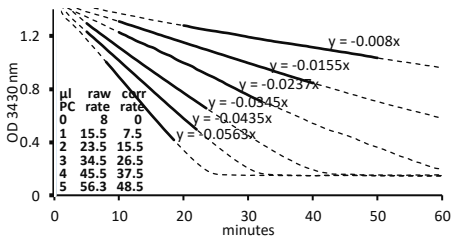
5. Initiate Reaction: Add 10 µl Cumene Hydroperoxide to test samples and positive control (NOT to the background control well) to start the reaction.

6. Measurement: Monitor the reaction kinetically at 25°C, at 340 nm long enough to establish a linear rate of reaction.
Notes: The initial OD at 340 nm in the test and positive control wells after 15-minute preincubation, should be greater than 1.0 prior to the addition of Cumene Hydroperoxide. If the reading is < 1.0, it means there is either too much GPx in the sample (dilute and rerun) or too much GSSG. High GSSG can be reduced by using a 10 kDa spin filter to remove small molecules from the sample.

7. Typical Results:

nmole Standard	Standard Raw Values OD		Background Corrected Values	
0	0.1511	0.1615	0	0
20	0.3834	0.3723	0.2323	0.2108
40	0.6152	0.6131	0.4641	0.4516
60	0.8337	0.8385	0.6826	0.6770
80	1.0555	1.0617	0.9044	0.9002
100	1.2717	1.2819	1.1206	1.1204





8. Calculation: Subtract the 0 Standard value from all other standards. Plot the Standard Curve. Determine the slope of the Standard Curve. Determine the slope of the background control and of the test wells. These typically have a slight sigmoidal shape due to an initial lag and a late slowing due to substrate depletion. Determine the slope of the linear middle portion of the test samples and subtract the slope of the background control to correct for non-GPx contributions. Divide the background corrected slope by the slope of the standard curve to get the sample slope in nmole/minute (mU) in the well. To convert to mU/sample:
A. Divide the mU/well by volume of sample applied to well = mU/µl sample.
B. Multiply mu/µl sample by total volume of supernatant obtained after centrifugation in step 2 above = total mU GPx/sample
C. Divide total PGx per sample by mg tissue or # of cells, etc. = mU GPX activity / mg (or # of cells, etc.).

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