ab102530 - Glutathione Peroxidase Assay Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of glutathione peroxidase activity in various samples.

View kit datasheet: www.abcam.com/ab102530

(use www.abcam.cn/ab102530 for China, or www.abcam.co.jp/ab102530 for Japan)

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Storage and Stabilty

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature. Reconstituted components are stable for 2 months.

Materials supplied

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Glutathione Peroxidase Assay Buffer	50 mL	-20°C	-20°C*
NADPH II/NADPH (lyophilized)	1 vial	-20°C	4°C or -20°C*
Active Glutathione Reductase/Glutathione Reductase	1 vial	-20°C	4°C or -20°C*
Glutathione/Glutathione (GSH; lyophilized)	1 vial	-20°C	4°C or -20°C*
Cumene Hydroperoxide	1 vial	-20°C	4°C or -20°C*
Active Glutathione Peroxidase/Glutathione Peroxidase Positive Control (lyophilized)	1 vial	-20°C	4°C or -20°C*

Materials required, not supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH$_2$O)
- PBS
- Colorimetric microplate reader – equipped with filter for OD340 nm
- 96 well plate: clear plates for colorimetric assay
- Microcentrifuge
- Pipettes and pipette tips
- Heat block or water bath
- Vortex
- Dounce homogenizer or pestle (if using tissue)

Reagent preparation

Briefly centrifuge small vials at low speed prior to opening.

Glutathione Peroxidase Assay Buffer:

Ready to use as supplied. Warm to room temperature before use. Store at -20°C.

NADPH Standard:

Reconstitute with 500 µL of dH$_2$O to get a 40 mM NADPH standard solution. Aliquot standard so that you have enough to perform the desired number of assays Store at -20°C for 1 month or at 4°C for 1 week.

Active Glutathione Reductase/Glutathione Reductase:

Dilute with 220 µL of Assay Buffer. Aliquot enzyme so you have enough to perform the desired number of assays. Store at -20°C for 1 month or at 4°C for 1 week. Keep on ice during use.

Glutathione/Glutathione (GSH):

Reconstitute with 220 µL of Assay Buffer. Aliquot GSH so that you have enough to perform the desired number of assays. Store at -20°C for 1 month or at 4°C for 1 week.

Cumene Hydroperoxide:

Dilute 5 µL with 3.125 mL of Assay Buffer. Aliquot so that you have enough to perform the desired number of assays. Store at -20°C for 1 month or at 4°C for 1 week.

Active Glutathione Peroxidase/Glutathione Peroxidase (Positive Control):

Reconstitute with 100 µL of Assay Buffer. Aliquot positive control so that you have enough to perform the desired number of assays Store at -20°C for 1 month or at 4°C for 1 week. Keep on ice during use.

Standard preparation

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

1. Prepare 1 mM NADPH Standard by diluting 25 µL of the 40 mM NADPH standard solution in 975 µL of dH$_2$O.
2. Using 1 mM NADPH standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End [NADPH] in well
1	0	300	100	0 nmol/well
2	60	240	100	20 nmol/well
3	120	180	100	40 nmol/well
4	180	120	100	60 nmol/well
5	240	60	100	80 nmol/well
6	300	0	100	100 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 100 µL).

Sample preparation

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest you complete the Sample Preparation step before storing the samples. Alternatively, you can snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware that this might affect the stability of your samples and the readings can be lower than expected.

Cell (adherent or suspension) samples and Tissue Samples:

1. Harvest the amount of cells necessary for each assay

- **Cell (adherent or suspension) initial recommendation** = 2 x 10^6 cells).
- **Tissue Samples initial recommendation** = 100 mg).

2. Wash cells/tissue with cold PBS.
3. Resuspend samples in 200 µL of cold Assay Buffer.
4. Homogenize samples quickly by pipetting up and down a few times, on ice.
5. Centrifuge 15 minutes at 4°C at 10,000g using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.

Erythrocytes:

1. Homogenize 200 µL erythrocytes in 200 µL cold Assay Buffer.
2. Centrifuge 15 minutes at 4°C at 10,000 x g using a cold microcentrifuge to remove any insoluble material.
3. Collect supernatant and transfer to a clean tube and keep on ice.

Plasma and serum samples:

Serum samples can be tested directly by adding sample to the microplate wells. Samples can be stored at -80°C.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

Assay Procedure And Detection

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

1. **Set up Reaction wells:**

- Standard wells = 100 µL standard dilutions.
- Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer).
- (Optional) Positive Control = 5 – 10 µL of the GPx Positive Control (adjust volume to 50 µL/well with Assay Buffer).
- Reagent Control wells = 50 µL Assay Buffer

2. **Reaction Mix:**

- Immediately prior to use, prepare Reaction Mix for each reaction:

Component	Colorimetric Reaction Mix (µL)
Assay Buffer	33
40 mM NADPH solution	3
GR solution	2
GSH solution	2

- Mix enough reagents for the number of to be performed. Prepare a master mix of the Reaction Mix to ensure consistency.

3. Add 40 µL of Reaction Mix to sample, positive control(s) and reagent control wells.
4. Mix well and incubate at room temperature for 15 minutes to deplete all GSSG in the samples.

NOTE*: Measure the OD 340 nm before adding cumene hydroperoxide. If the OD at 340 nm is lower than 1.0 add more NADPH to ensure there is enough NADPH in the reaction system. 1 µL of 40 mM NADPH will give ~0.5 OD at 340 nm.*

5. Add 10 µL cumene hydroperoxide solution, to the sample, positive control and reagent control wells only, to start the glutathione peroxidase (GPx) reaction. Mix well.
6. Measure output (A_1) on a microplate reader at OD340 nm at T_1.
7. Incubate at 25°C for 5 min (or longer if the GPx activity is low). Protect from light.
8. Measure output (A_2) on a microplate reader at OD340 nm at T_2.

NOTE: *If A_1 reading is too low (<0.7), it means either too much GPx or too much GSSG is present 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 (ab93349) to remove GSSG.*

NOTE: *It is essential to read A_1 and A_2 in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose A_1 and A_2 in the reaction linear range.*

Calculations

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
3. Plot the corrected absorbance values for each standard as a function of the final concentration of NADPH.
4. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
5. Extrapolate sample readings from the standard curve plotted using the following equation:

$$\Delta A_340nm = ((Sample\ A1 - Sample\ A2) - (Reagent\ control\ A1 - Reagent\ control\ A2))$$

6. Apply the Δ_A340nm to the NAPDH standard curve to get NADPH amount B:

$$B = \left(\frac{\Delta A_340nm - intercept}{Slope}\right)$$

7. Concentration of GPx in the test samples is calculated as (nmol/min/mL = mU/mL):

$$GPx\ Activity = \left(\frac{B}{(T_2 - T_1) * V}\right) * D$$

Where:

B = NADPH amount that was decreased between T_1 and T_2 (in nmol).
T_1 = Time of the first reading (A_1) (minutes).
T_2 = Time of second reading (A_2) (minutes).
V = Pretreated sample volume added into the reaction well (mL).
D = Sample dilution factor.

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°C.

Quick assay procedure

NOTE*: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare NADPH standard, Active Glutathione Reductase/glutathione reductase, GSH, cumene hydroperoxide, GPx positive control, (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve.
- Set up plate for standard (100 µL) and samples (50 µL), positive control (50 µL), and reagent control wells (50 µL), in duplicate. Find optimal dilutions to fit standard curve readings.
- Prepare Reaction Mix (Number of samples + 1).

Component	Colorimetric Reaction Mix (µL)
Assay Buffer	33
40 mM NADPH solution	3
GR solution	2
GSH solution	2

- Add 40 µL Reaction Mix to sample, positive control and reagent control wells only.
- Incubate plate RT 15 mins.
- Read at OD 340 nm.
- Add 10 µL cumene hydroperoxide solution to sample, positive control and reagent control wells only.
- Measure output (A1) on a microplate reader at T1 at OD340 nm.
- Incubate at 25°C for 5 min, protected from light (or longer).
- Measure output (A2) on a microplate reader at T2 at OD340.

Troubleshooting

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

FAQ

What is the minimum detection of this kit?

The assay has a detection sensitivity of 0.5 mU/mL of glutathione peroxidase in samples.

Can this kit be used with plasma and whole blood?

The protocol contains instructions for erythrocytes. Whole blood can be processed similarly. Plasma can be diluted over a range and then the dilution that gives readings within the linear range of the standard curve can be used for the assay.

What is the activity level of the positive control? How can we increase its value to be comparable with our samples?

The positive control is only a benchmark sample. As long as the values are within the range of the standard curve this is fine. The positive control is not be used to compare values with the samples. The positive control is provided to validate that the assay components are all working.

Technical Support

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