Reduced Glutathione (GSH) Colorimetric Assay Kit

Catalog Number EEA020 (96 tests)

Rev 2.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

This kit can measure GSH content in serum, plasma, cells, cell culture supernatant and tissue samples.

Reduced Glutathione (GSH) is a type of low molecular scavenger, which can remove O_2^-, H_2O_2, and lipid hydroperoxides. GSH is a small molecule peptide which is composed of glutamic acid, glycine, and cysteine, and is the main thiol compound of non-protein in the organism. GSH is the substrate of GSH-Px and GSH-ST, which is indispensable for decomposing hydrogen peroxide of these two enzymes. It can stabilize the enzyme containing thiol and prevent hemoglobin and other auxiliary factors from the oxidative damage. Recently, it has been proven that GSH is also involved in the recovery of vitamin E to the reduction state. When biological systems are lacking in GSH, it may induce toxic effects or increase the toxic effects of many chemicals or environmental factors. It may be related to the increase of oxidative damage, so the amount of GSH is a vital factor to measure the body's antioxidant ability. GSH plays an important role in the research processes of prevention, recovery and treatment of atherosclerosis, coronary heart disease, anti-aging, anti-tumor, prevention, prevention of Alzheimer's disease and other diseases.

Reduced GSH can react with Dinitrobenzoic acid (DNTB) to form a yellow complex which can be detected by colorimetric assay at 405 nm to calculate the reduced GSH content indirectly.

Contents and storage

Kit and components are shipped at 2-8℃. An unopened kit can be stored at 2-8℃ for 12 months.

Components	Quantity (96 tests)
Acid Reagent	12 mL
Phosphate Solution	12 mL
DTNB Solution	1.5 mL ×2 vials
GSH Standard	3.07 mg ×2 vials
GSH Standard Stock Diluent	1.5 mL ×2 vials
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)
- Microplate reader (405-414 nm)
- Micropipettor
- Vortex mixer

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Sample requirements:

DTT, 2-mercaptoethanol and other reductive substances should not be added in the samples.

Serum and plasma samples: Detect the sample directly. If the sample is turbid, centrifuge at 10000 g for 10 min at 4 ℃, then take the supernatant for detection.

Tissue sample:

- Take 0.02-1 g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8 ℃ to remove blood cells.
- Absorb the water with filter paper and weigh.
- Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) (2-8 ℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4 ℃.
- Take the supernatant and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).

If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for 1 month.

Cells:

- Take the supernatant and preserve it on ice for detection.
- Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) 1 time.
- Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment.
- Add homogenization medium at a ratio of cell number (10^6): PBS (0.01 M, pH 7.4) (μL) =1: 300-500.
- Homogenize cells by an ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min) or homogenizer instrument (60 Hz 90s) on ice.
- Centrifuge at 10000 g for 10 min at 4 ℃, then take the supernatant and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).

If not detected on the same day, the cells sample (without homogenization) can be stored at -80 ℃ for 1 month.

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (2-100 μmol/L).

Note: Use all samples within 2 hours of dilution

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

Sample type	Dilution factor
Human serum	1
Human plasma	1
10% Mouse brain tissue homogenization	1
10% Mouse liver tissue homogenization	1
Hela cell homogenization (0.999 mgprot/mL)	1
Rat serum	1
Rat plasma	1
Mouse serum	1
10% Carrot tissue homogenization	1
293T supernatant	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Preparation of GSH standard diluent

Dilute the GSH standard stock diluent with distilled water at a ratio of 1:9. Prepare the fresh solution before use.

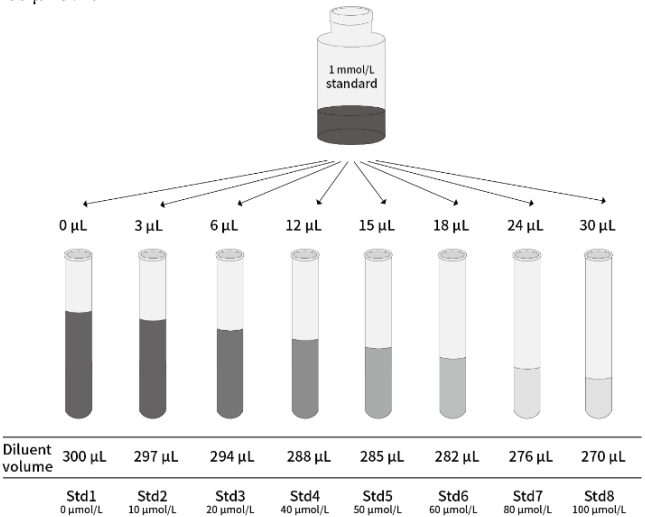
Preparation of 1 mmol/L GSH standard solution

Dissolve 3.07 mg of GSH standard with 10 mL of GSH standard diluent and mix fully. Prepare the fresh solution before use. The unused solution can be aliquoted into smaller quantities and stored at -20℃ for 1 month.

Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

Dilute 1 mmol/L GSH standard solution with GSH standard diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 μmol/L.



Assay Protocol

1. Preparation of sample supernatant: take 0.1 mL of sample, add 0.1 mL of acid reagent and mix fully. Centrifuge at 4500 g for 10 min. Take the supernatant for detection.
2. Add 25 μL DTNB solution to each tube.
3. Control well: Add 100 μL of acid reagent.
4. Standard well: Add 100 μL of standard solutions with different concentrations.
5. Sample well: Add 100 μL of supernatant.
6. Add 100 μL of phosphate solution to each tube.
7. Mix fully for 1 min and stand for 5 min at room temperature. Measure the OD values of each well at 405 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Std1) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

Serum (plasma) and other liquid sample:

$$\text{GSH content (μmol/L)} = (\Delta A_{405}\text{-b}) \div a \times 2^* \times f$$

Tissue and cells sample:

$$\text{GSH content (μmol/gprot)} = (\Delta A_{405}\text{-b}) \div a \times 2^* \times f \div C_{pr}$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0);
x: The concentration of standard;
a: The slope of standard curve;
b: The intercept of standard curve.
ΔA_{405}: $OD_{Sample} - OD_{Control}$.
2*: Dilution factor of in the preparation step of sample supernatant, 2 times;
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample (gprot/L)

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

Take 0.1 mL of human serum sample, add 0.1 mL of acid reagent, mix fully and centrifuge at 4500 g for 10 min, then take prepared supernatant, carry the assay according to the operation steps. The results are as follows:

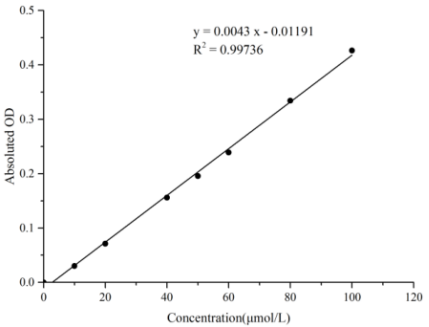
standard curve: y = 0.00383x – 0.00251, the average OD value of the sample is 0.080, the average OD value of the control is 0.047, and the calculation result is:

$$\text{GSH content (μmol/L)} = (0.080 - 0.047 + 0.00251) \div 0.00383 \times 2 = 18.54 \text{ μmol/L}$$

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 0–100 µmol/L standard.

Concentration (µmol/L)	OD at 405 nm	Standard Curve
100.00	0.514	
80.00	0.413	
60.00	0.332	
50.00	0.293	
40.00	0.244	
20.00	0.164	
10.00	0.120	
0.00	0.086	

- Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	5.50	26.40	64.50
%CV	2.3	2.0	1.4

CV = Coefficient of Variation

- Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	5.50	26.40	64.50
%CV	3.5	2.9	3.2

CV = Coefficient of Variation

- Expected values

This assay was tested with human serum, and plasma samples at dilutions from 1:10 to 1:60 in Assay Buffer.

Sample Type	Range (µmol/L)	Average (µmol/L)
Human Serum	15-26	23.3
Human plasma	13-23	18.96

- Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 96%.

	Standard 1 (low conc.)	Standard 2 (middle conc.)	Standard 3 (high conc.)
Expected Conc. (μmol/L)	15	45	58
Observed Conc. (μmol/L)	14.9	42.3	55.1
Recovery rate (%)	99	94	95

- Recommended Plate Set Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80
[Note]: A-H, standard wells; S1, Control wells; S2-S80, sample wells.												

- Sensitivity

The analytical sensitivity of the assay is 2 µmol/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.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support.**

Corporate entity: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.