Oxidized Glutathione (GSSG)Assay Kit

Cat No. GSSG-M-50
Size: 50 Reactions
Store at 4°C

MOLEQULE-ON®

Components	Quantity
Solution I	50 ml
Solution II	170 µl
Solution III	60 ml
Solution IV	8 ml
Solution V[1]	Powder
Solution VI[2]	40 µl
Standard	10 mg

Description

Oxidized Glutathione (GSSG) is an oxidized form of glutathione (GSH), also known as dithione glutathione, formed by the oxidation of two molecules of glutathione. GSSG is reduced to GSH by glutathione reductase, so most of the body is in the reduced form. The determination of GSH and GSSG content and ratio of GSH/GSSG in cells can reflect the redox status of cells. This kit utilizes reaction of glutathione and (5, 5'-dithiobis-2-nitrobenoic acid, DTNB) to produce 5-thio-2-nitrobenzoic acid, 5-thio-2-nitrobenzoic acid has the largest absorption at wavelength of 412 nm ,and 2-Vinylpyridine inhibit reduced glutathione in the original of samples, and then using glutathione reductase to reduce GSSG to GSH, determining the content of Oxidized Glutathione.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, benchtop centrifuge, micropipette, mortar, Analytical balance, homogenizer (2ml), water bath, 1ml cuvette.

Preparation:

- [1] ***Working solution V: dissolve into 8ml distilled water, then aliquot into smaller volume***
- [2] ***Working solution VI: dissolve into 0.8ml ddH$_2$O***

Protocol

I. Sample preparation:

1. Tissue sample

Wash fresh tissues with PBS for twice, then add 0.1g sample into homogenizer(Before use, wash homogenizer with Solution I place on ice). Add 1ml Solution I(the proportion of tissue and reagents can be kept constant), fully grinding on ice(use liquid nitrogen will have a better grinding effect); 8000g 4℃ centrifuge 10min. Place supernatant at 4 ℃ for test. (The supernatant can be stored at -80℃ for 10 days).

2. Blood sample

Plasma: Sample centrifuge at 600g for 10min, 4℃. Absorb the upper plasma into another tube add with same volume Solution I, centrifuge at 8000g for 10min, 4℃. Place supernatant at 4 ℃ for test. (The Supernatant can be stored at -80℃ for 10 days.)

Blood cell: Sample centrifuge at 600g for 10min, 4℃. Discarding the upper plasma, wash with treble volume of PBS for 3 times(mix blood cell with PBS centrifuge at 600g for 10min), add equal volume of Solution I. Centrifuge at 8000g for 10min. Place supernatant at 4 ℃ for test. (The supernatant can be stored at -80℃ for 10 days).

3. Cell sample

Harvest cell should not less than 108 then wash with PBS for twice (mix cell with PBS centrifuge at 600g for 10min), wash with treble volume of PBS for 3 times. Repeated freezing and thawing 2-3 times (suggest frozen in liquid nitrogen, dissolved in 37 ℃ water bath). Centrifuge at 8000g for 10min. Place supernatant at 4 ℃ for test. (The supernatant can be stored at -80℃ for 10 days.)

II. Determination procedure:

1. Preheat spectrophotometer for 30 min, adjust the wavelength to 412nm, set the counter to zero with ddH_2O.
2. Preheat Solution Ⅱ in water bath: 37℃ (mammal cell) , 25℃ (other species).
3. The standard dilution: dissolve standard in 1 ml of distilled water (4 ℃) to concentration of 10 mg/ml. Take suitable solution to prepare the standard of concentration of 25μg/ml, 20 μg/ml, 12.5μg/ml, 6.25 μg/ml, 3.125 μg/ml, and 0 μg/ml (The diluent is a ten-fold diluted Solution I).
4. Add 100 ml diluted standard or sample to 1.5 ml centrifuge tube, add 2 ul Solution Ⅱ, mix well, incubate at 37℃ for 30 min.
5. Make standard curve.
6. After the incubation, add 700 μl Solution Ⅲ, 100 μl Solution Ⅳ, 100 μl Solution Ⅴ, and 10 μl Solution Ⅵ to the standard tube in sequence. After rapid mixing, the light absorption A1 and A2 of 30s and 150s respectively were measured at 412nm. Absorbance (A2-A1) is the abscissa (x) and concentration is the ordinate (y), making the standard curve.
7. Add 700 μLlSolution Ⅲ, 100 μl Solution Ⅳ, 100 μl Solution Ⅴ, and 10 μl Solution Ⅵ to the sample tubes in sequence. After rapid mixing, the light absorption A1 and A2 of 30s and 150s respectively were measured at 412nm, ΔA= A2- A1.

Calculations

According to the standard curve, sample ΔA into the formula (x), calculate the sample concentration of Y (μg/ml).

Protein concentration

GSH (μg / mg prot) = y×Vrv÷Vrv÷Cpr =y÷Cpr

Sample weight

GSH (μg /g) = y×Vrv÷(Vrv÷Vsv×W) = y÷W

Cell amount

GSH (μg /104 cell) = y×Vrv÷(Vrv÷Vsv×n) = y÷n

Solution volume

GSH (μg / mL) = 2y

n: Cell amount

Vsv: Total supernatant volume, 1 ml ;

Vrv: Supernatant volume added into the reaction system, 100μl=0.1 ml ;

W: Sample weight, g;

Cpr: Supernatant protein concentration, mg/ml.

Note:

The sample needs to be treated completely. If the test cannot be completed temporarily, it can be stored at -80℃.

If the GSSG content in the sample is uncertain, Dilute the sample for several gradients before test.

The substrate concentration was calculated by the method of enzymatic reaction rate, and the reading was completed in 30 seconds and 150 seconds as accurately as possible.

This method uses the enzymatic reaction rate to calculate the substrate concentration and complete readings as accurately as possible at 30s and 150s.

The supernatant could not be used for protein concentration determination.