GENLISA™ Reduced Glutathione (GSH) Assay

REF : KBCA2066

Ver 1.1

RUO

Biochemical Assay for the Quantitative Determination of Reduced Glutathione (GSH) in serum, plasma tissue cells, cell culture supernatants and other biological samples.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBCA2066 Σ 96 tests

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Introduction:

Glutathione (GSH) is a low molecular mundificant which can get rid of O2-, H2O2 & LOOH. GSH is a tripeptide composed by glutamic acid, glycine & cysteine, it is main non-protein sulfhydryl compound in tissue and substrate of GSH-PX & GST (GSH-PX & GST need GSH to decompose hydroperoxides). GSH can also stabilize sulfhydryl containing enzymes and avoid oxidative damage of hemoglobin & other cofactors. Recently, GSH is proved to participate function of recovering vitamin E to reduction form. Absence or exhausting of GSH will induct various chemicals or environmental factors to cause or aggratate toxication, it may relate to increasing oxidative damage, so GSH content is an important factor to measure oxidizing ability in vivo.

Intended Use

The GENLISA™ Reduced glutathione (GSH) Assay kit is used as an analytical tool for quantitative determination of Reduced glutathione (GSH) in serum, plasma, tissue cells, cell culture supernatants and other biological samples.

Principle:

Dithio-dinitrobenzoic acid can react with sulfhydryl compounds to produce a yellow compound, it can be quantitative estimated by colorimetric method at 405nm.

Materials Provided:

1. Reagent 1 (Precipitant) – 1 vial x 20 ml
2. Reagent 2 (Buffer) – 1 vial x 20 ml
3. Reagent 3 (Chromogenic agent) – 1 vial x 5 ml
4. Reagent 4 (GSH standard - lyophilized) – 3 vials x 3.07mg
   (Standard solvent stock solution) – 1 vial x 10 ml
5. Microwell Plate (96 wells) - 1 no.

Handling/Storage:

1. All reagents should be stored as indicated on the component label and keep away from the light
2. All the reagents should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Materials & Equipment Required But Not Provided

1. Microplate Reader/Visible Spectrophotometer
2. Incubator
3. Refrigerated
4. Centrifuge
5. Ice maker
6. 96 well Plate
7. Pipettes
8. Deionized Water
9. Homogenizer (for Tissue Samples)

Sample Preparation

1. **Cultured cells:** Rinse collected cells by PBS 1~2 times, do low speed centrifugation to get cell sediment, add 0.3 ～0.5ml 0.1M isotonic PBS (pH7.4) to make cell suspension, disrupt cells by ultrasonication or hand-driven.

   Supernatant preparation: Take cracked cell suspension 0.1ml,and then add 0.1ml Reagent 1,mix well.and then centrifugate at 3500rpm for 10 minutes, take supernatant for assay.

2. **Tissues:** Weigh sample accurately, add 9 times volume physiological saline according to mass (g)-volume (ml) ratio of 1:9, make homogenate in ice water bath, centrifugate at 2500rpm for 10 minutes, take supernatant for assay.

   Supernatant preparation: Take tissue homogenate 0.1ml, and then add 0.1ml Reagent 1,mix well.and then centrifugate at 3500rpm for 10 minutes, take supernatant for assay.

3. **Serum, blood plasma**：Take 0.05ml serum (blood plasma), add 0.2ml Reagent 1 working solution, mix sufficiently, centrifugate at 3500~4000rpm for 10 minutes. Take 1ml supernatant forassay.

4. **Whole blood**:

   10% hemolysate preparation: Take 0.1ml heparin anticoagulated whole blood, add 0.9ml double distilled water, mix sufficiently until hemolysate becomes limpid.

   Supernatant preparation: Take 10% hemolysate 0.05ml, and then add 0.2ml Reagent 1,mix well.and then centrifugate at 3500rpm for 10 minutes, take supernatant for assay.

Reagent Preparation

GSH standard solvent working solution preparation: When use, dilute standard solvent stock solution with double distilled water at ratio of 1:9, consider solution volume according to your need, working solution should be used soon after preparation.

1mmol/L GSH standard solution preparation: GSH's molecular weight is 307, add 3.07mg GSH standard powder in 10ml GSH standard solvent working solution, mix sufficiently, can be stored at 2^0C～8^0C for 48 hours.

20umol/L GSH standard solution preparation: Transfer 0.2ml 1mmol/L GSH standard solution in 9.8ml GSH standard solvent working solution. Please use this solution soon after preparation.

Assay Procedure:

	Blank Tube	Standard Tube	Sample Tube
Reagent 1 (ul)	100		
20umol/l GSH standard (ul)		100	
Supernatant (ul)			100
Reagent 2 (ul)	100	100	100
Reagent 3 (ul)	25	25	25
Mix sufficiently, place for 5 minutes, transferto microplate measure OD values at 405nm			

Procedural Notes:

1. Wear appropriate personal protective equipment including lab coats, eye protection, and latex gloves. Adhere to all national security protocols for biological laboratories, particularly when handling blood samples or other bodily fluids.

2. Do not mix or use components from different lots. The kit must not be used beyond the expiration date printed on the kit label.
3. To avoid cross-contamination, change pipette tips between each reagent addition. Additionally, use separate reservoirs for each reagent.
4. Briefly centrifuge small vials at low speed before opening.
5. Fresh samples are recommended. If not assayed immediately, samples should be stored at -80°C for up to one month.
6. Always prepare fresh Standards for each use. Diluted Standard solutions are unstable and must be used within 4 hours of preparation.

4. Formula:

(1) Whole Blood:

$$\text{GSH Content in Whole Blood (umol/l)} = \frac{\text{OD (Sample)} - \text{OD (Blank)}}{\text{OD (Standard)} - \text{OD (Blank)}} \times \text{Standard concentration (20 umol/l)} \times \text{dilution ratio before sample process (5X)} \times \text{Dilution ratio before Sample Test (10X)}$$

(2) Cell Tissue:

$$\text{GSH Content in cell and tissue (umol/l)} = \frac{\text{OD (Sample)} - \text{OD (Blank)}}{\text{OD (Standard)} - \text{OD (Blank)}} \times \text{Standard concentration (20 umol/l)} \times \text{dilution ratio before sample process (2X)} \times \text{Homogenate protein concentration before test (gprot/l)}$$

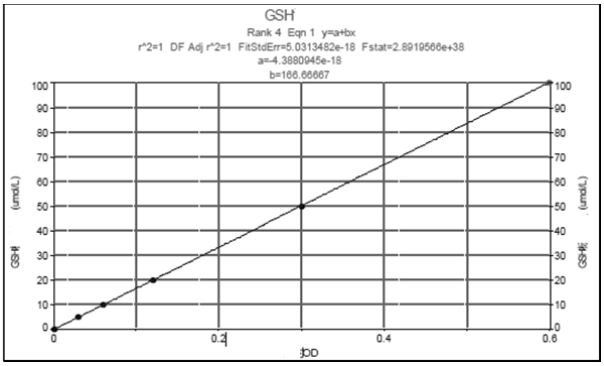
(3) Serum (Blood Plasma):

$$\text{GSH Content in Serum/plasma (umol/l)} = \frac{\text{OD (Sample)} - \text{OD (Blank)}}{\text{OD (Standard)} - \text{OD (Blank)}} \times \text{Standard concentration (20 umol/l)} \times \text{dilution ratio before sample process (5X)}$$

5. Standard Curve: (The sensibility is 1.5umol/l)

Dilute 1mmol/l GSH standard solution to: 100umol/l, 50umol/l, 20umol/l, 10umol/L, 5umol/l, 0umol/l to assay for Standard curve.

Typical Graph



Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

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