GENLISA™ Superoxide Dismutase (SOD) Assay (WST-1 method)

REF : KBCA2166

Ver 1.1

RUO

Quantitative Biochemical Assay for the Determination of Superoxide Dismutase (SOD) in serum or plasma 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 KBCA2166 Σ 96 tests

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Introduction:

The GENLISA™ Biochemical Assays kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs an established biochemical method which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

Principle:

A Superoxide Dismutase (SOD) assay measures the activity of the SOD enzyme by detecting the decrease in superoxide anions. SOD is an antioxidant enzyme that converts superoxide radicals into hydrogen peroxide and oxygen.

Materials Provided:

1. Reagent 1 (Buffer) – 1 x 30 ml
2. Reagent 2 (Substrate Stock Solution, concentrated) – 1 x 0.15 ml
3. Reagent 3 (Enzyme Stock Solution, concentrated) – 1 vial x 0.3 ml
4. Reagent 4 (Enzyme Diluent) – 1 x 4 ml

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.

Reagent Preparation

Reagent 2 (Substrate Stock Solution):
Dilute **Substrate Stock Solution** with buffer in a ratio of 1:200. Mix well. This **Working Substrate Solution** should be used immediately after preparation. Prepare the **Working Substrate Solution** according to your needs. The residual Working Substrate Solution may be stored at 2-8ºC for 3 days.

Reagent 3 (Enzyme Stock Solution):
Dilute **Enzyme Stock Solution** with Enzyme Diluent in a ratio of 1:10. Mix well. This **Working Enzyme Solution** should be used immediately after preparation. Prepare the **Working Enzyme Solution** according to your needs. The residual Working Enzyme Solution may be stored at 2-8ºC for 3 days.

Required Equipments & Reagents

- An enzyme-labeled instrument capable of measuring absorbance at 450nm
- 96-wells microwell plate
- Pipettes (single channel & multichannel)
- Thermostatic incubator

Note:

1) It is recommended to use a multichannel micropipet to add Working Substrate Solution. This will reduce the operational assay time leading to lower errors between different wells.
2) Use a 96-well microplate to run your assay. Mix well.
3) Before starting the assay, please choose 1~2 normal group samples. Dilute them to different
4) concentrations to do pre-test. Choose the concentrations whose inhibition percentage is
5) between 40%~60%. We recommend to then start the actual assay. C
6) This method has a high specificity for SOD analysis, as it removes disturbances of SOD analog chemicals. If you wish to measure the SOD analog chemicals, then you purchase a SOD Analogs Assay kit from us.
7) Inhibition percentage can reach 100% by using this method.
8) It only needs 1~2 contrast, 1~2 contrast blank and 1~2 sample blank wells for each lot of experiments.
9) The kit is for laboratory and research use only. It is not fo diagnostic use.

Unit Definition:

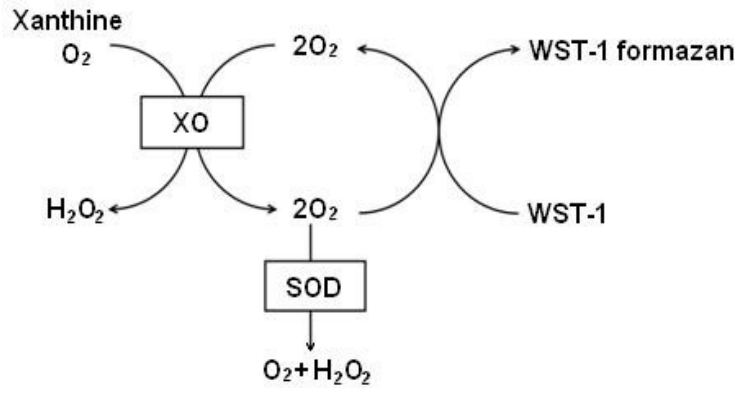
Unit Definition: Corresponding quantity of SOD that its inhibition pecentage reaches 50% in this reaction system is considered as one SOD activity unit (U).

SOD activity unit has different difinitions in different experimental conditions, for example, samples of blood serum (or plasma), tissue, cultured cells, erythrocytes, plants, medines, cosmetics, drinks, etc. are different. Therefore please review the different quantification formulas based on your sample type in the Appendix below.

Key Technical Parameters:

- Contrast OD: ≥ 0.2
- Reaction Temperature: 37ºC
- Wavelength: 450nm
- Precision: Inter-Assay: 5.05%
- Intra-Assay: 3.32%
- Detection Limit: 0.5U/ml

Assay Principle:



Sample Pretreatment:

(1) Check blood serum (or plasma) sample, if it is turbid, then centrifugate at 3500rpm for 10 minutes. Take the supernatant for the assay.

(2) Dilute limpid blood serum (or plasma) sample with physiological saline to different concentrations in order to do the pre-test.

Assay Procedure:

Reagent \ Well	Contrast Well	Contrast Blank Well	Sample Well	Sample Blank Well
Sample	-	-	20ul	20ul
Double distilled water	20ul	20ul	-	-
Working Enzyme Solution	20ul		20ul	-
Enzyme Diluent	-	20ul	-	20ul
Working Substrate Solution	200ul	200ul	200ul	200ul
Mix well. Incubate at 37ºC for 20 minutes. Measure the absorbances at 450nm.				

Calculation of Results:

Definition: Corresponding quantity of SOD that its inhibition pecentage reaches 50% in this assay is considered as one SOD activity unit (U).

SOD Inhibition Percentage Calculation:

$$\text{SOD inhibition percentage} = \frac{(A_{\text{Contrast}} - A_{\text{Contrast blank}}) - (A_{\text{Sample}} - A_{\text{Sample blank}})}{(A_{\text{Contrast}} - A_{\text{Contrast blank}})} \times 100\%$$

SOD Activity Calculation:

$$\text{SOD activity (U/ml)} = \text{SOD inhibition percentage} \div 50\% \times \text{Reaction system dilution times} \left(\frac{0.24ml}{0.02ml}\right) \times \text{Sample dilution times before assay}$$

Example:

Take 20ul normal human blood plasma sample (already 5 times diluted with physiological saline). Run the assay as per the Assay Protocol.. In results, Contrast is 0.3214, Contrast blank is 0.0765, Sample is 0.2418, Sample blank is 0.1031, calculate as follows:

$$\text{SOD inhibition percentage} = \frac{(A_{\text{Contrast}} - A_{\text{Contrast blank}}) - (A_{\text{Sample}} - A_{\text{Sample blank}})}{(A_{\text{Contrast}} - A_{\text{Contrast blank}})} \times 100\%$$

$$= \frac{(0.3214 - 0.0765) - (0.2418 - 0.1031)}{(0.3214 - 0.0765)} \times 100\% = 43.36\%$$

$$\text{SOD activity (U/ml)} = \text{SOD inhibition percentage} \div 50\% \times \text{Reaction system dilution times} \left(\frac{0.24ml}{0.02ml}\right) \times \text{Sample dilution times before assay}$$

$$= 43.36\% \div 50\% \times \frac{0.24ml}{0.02ml} \times 5 = 52.03(U/ml)$$

Animal Tissue Sample SOD Assay

Sample Pretreatment:

(1) Weigh sample accurately, add 9 times volume physiological saline according to mass(g)-volume(ml) ratio of 1:9, cut tissue to small pieces, make homogenate in ice water bath. Centrifugate at 2500~3000rpm for 10 minutes, take supernatant (as 10% homogenate supernatant) for assay.
(2) After sample preparation above, you can use BCA kit or Coomassie brilliant blue kit to measure protein concentration in sample, it will be used in calculation.

Example:

Take 20ul 10% mouse brain tissue homogenate sample (already 100 times diluted with physiological saline), Run the assay as per the Assay Protocol. In results, Contrast is 0.3214, Contrast blank is 0.0765, Sample is 0.2047, Sample blank is 0.0788, protein concentration in 1% brain homogenate is 0.435mg prot/ml,

Calculate as follows:

$$\text{SOD inhibition percentage} = \frac{(A_{\text{Contrast}} - A_{\text{Contrast blank}}) - (A_{\text{Sample}} - A_{\text{Sample blank}})}{(A_{\text{Contrast}} - A_{\text{Contrast blank}})} \times 100\%$$

$$= \frac{(0.3214 - 0.0765) - (0.2047 - 0.0788)}{(0.3214 - 0.0765)} \times 100\% = 48.59\%$$

$$\text{SOD activity (U/mgprot)} = \text{SOD inhibition percentage} \div 50\% \times \text{Reaction system dilution times} \left(\frac{0.24ml}{0.02ml}\right) \times \text{Protein concentration in sample to assay (mgprot/ml)}$$

$$= 48.59\% \div 50\% \times \frac{0.24ml}{0.02ml} \div (0.435 \div 10) = 268.08(U/mgprot)$$

Cultured Cells SOD Assay

1. Pretreatment:

(1) Collection of cultured cells:

Suspension cultured cells: Can be collected by centrifugation directly (centrifugate at 1000 rpm for 10 minutes, remove supernatant and take sediment cells).

Adherent cultured cells: Can be scraped down by cell scraper directly after removing supernatant. They can also be digested by 0.25% trypsogen at room temperature for 2~3 minutes, add culture solution to terminate digestion, use micropipet to blow slightly, transfer all liquid in EP tube, then centrifugate at 1000rpm for 10 minutes, remove supernatant and take sediment cells, add 1ml PBS and blow sligtly again, centrifugate at 1000rpm for 10 minutes, remove supernatant and keep sediment cells to use. If you don't want to assay immediately, cells can be cold preservated at low temperature, the colder, the better.

(2) Disintegration of cultured cells:

Grinding disruption: Add a certain amount of buffer (for example, 10^6 cells with 0.3~0.5ml buffer) such as PBS or physiological saline, use hand-move glass homogenizer to grind in icewater bath for 3~5 minutes, or use Kflou motor-operated grinder to grind in icewater bath for 3 minutes to assay;

Ultrasonication: Add a certain amount of buffer (10^6 cells with 0.3~0.5ml buffer) such as PBS or physiological saline, keep ultraphonic probe under liquid level. Ultrasonication power is 300W, place tubes in icewater bath, do Ultrasonication every 3~5 seconds, there is 4 intervals and each interval's length is 30 seconds.

Repeatedly freeze-thawing: Liquid nitrogen can be used to repeatedly freeze thawing, transfer cells to EP tube or freezing storage tube, add certain amount of hypotonic solution or double distilled water, put tube in liquid nitrogen directly for 3~5 seconds, take tube out of liquid nitrogen immediately, keep it at -20℃ in fridge for 20~30 seconds, then put it at room temperature to thaw, repeat these steps 3 times. (Note: Don't put tube at temperature after take it out of liquid nitrogen or EP tube will easily split and cause loss of sample, as the result, gradient thawing must be used, -20℃ step cannot be skipped.)

Chemical disruption: For adherent cultured cells, remove supernatant directly, add certain amount of lysis buffer into wells or tubes (flood cells completely). Keep lysis for 30~40 minutes (you may check lysis situation by microscope), then use micropipet to transfer some suspension to assay. According to real conditions, samples can be diluted by physiological saline or PBS.

(3) After sample preparation above, you can use BCA kit or Coomassie brilliant blue kit to measure protein concentration in sample, it will be used in calculation.

SOD Inhibition Percentage Calculation:

$$\text{SOD inhibition percentage} = \frac{(A_{\text{Contrast}} - A_{\text{Contrast blank}}) - (A_{\text{Sample}} - A_{\text{Sample blank}})}{(A_{\text{Contrast}} - A_{\text{Contrast blank}})} \times 100\%$$

SOD Activity Calculation:

$$\text{SOD activity (U/mgprot)} = \text{SOD inhibition percentage} \div 50\% \times \text{Reaction system dilution times} \left(\frac{0.24ml}{0.02ml}\right) \times \text{Protein concentration in sample to assay (mgprot/ml)}$$

Example:

Digest cells by trypsogen, wash cells once with physiological saline, centrifuge, remove supernatant and keep sediment, add 300ul physiological saline to make cell suspension, use motor-operated grinder to grind cells in icewater bath, dilute suspension 10 times with physiological water, take 20ul diluted suspension to operate according to table above. In results, Contrast is 0.3214, Contrast blank is 0.0765, Sample is 0.2016, Sample blank is 0.0793, protein concentration in cell stock suspension is 0.5208mg prot/ml, calculate as follows:

$$\text{SOD inhibition percentage} = \frac{(A_{\text{Contrast}} - A_{\text{Contrast blank}}) - (A_{\text{Sample}} - A_{\text{Sample blank}})}{(A_{\text{Contrast}} - A_{\text{Contrast blank}})} \times 100\%$$

$$= \frac{(0.3214 - 0.0765) - (0.2016 - 0.0793)}{(0.3214 - 0.0765)} \times 100\% = 50.06\%$$

$$\text{SOD activity (U/mgprot)} = \text{SOD inhibition percentage} \div 50\% \times \text{Reaction system dilution times} \left(\frac{0.24ml}{0.02ml}\right) \times \text{Protein concentration in sample to assay (mgprot/ml)}$$

$$= 50.06\% \div 50\% \times \frac{0.24ml}{0.02ml} \div (0.5208 \div 10) = 230.6912 (U/mgprot)$$

Plant Tissue SOD Assay

Sample pretreatment:
Weigh plant tissue to assay accurately, cut tissue to small pieces, dilute plant tissue with 0.1mol/L pH 7~7.4 phosphate buffer at mass-weight ratio of 1:9 (for example, plant tissue : 0.1mol/L pH 7~7.4 phosphate buffer = 1g : 9ml) to make homogenate by homogenizer or homogenate tube, centrifugate at more than 3500rpm or higer speed for 10 minutes, take supernatant as 10% homogenate, dilute homogenate supernatant with phosphate buffer to different concentrations in order to do pre-test.

Note: You can increase centrifuging speed and time properly in order to make plant homogenate supernatant as limpid as possible.

Formulas for Plant Tissue Samples:

SOD Inhibition Percentage Calculation:

$$\text{SOD inhibition percentage} = \frac{(A_{\text{Contrast}} - A_{\text{Contrast blank}}) - (A_{\text{Sample}} - A_{\text{Sample blank}})}{(A_{\text{Contrast}} - A_{\text{Contrast blank}})} \times 100\%$$

SOD Activity Calculation:
By protein concentration:

$$\text{SOD activity (U/mgprot)} = \text{SOD inhibition percentage} \div 50\% \times \text{Reaction system dilution times} \left(\frac{0.24ml}{0.02ml}\right) \times \text{Protein concentration in sample to assay (mgprot/ml)}$$

By tissue weight:

$$\text{SOD activity (U/g tissue)} = \text{SOD inhibition percentage} \div 50\% \times \text{Reaction system dilution times} \left(\frac{0.24ml}{0.02ml}\right) \times \text{Sample dilution times before assay} \div \frac{\text{Sample weight (g)}}{\text{Added buffer volume(ml)}}$$

Examples:

Take 0.2g leaf tissue accurately, add 1.8 ml 0.1M phosphate buffer, cut tissue to small pieces, make 10% leaf homogenate in icewater bath, dilute this homogenate 10 times by phosphate buffer, take 20ul diluted homogenate and run the assay as per the Assay Protocol showin herein. In results, Contrast is 0.3214, Contrast blank is 0.0765, Sample is 0.2575, Sample blank is 0.0791, protein concentration in 10% leaf homogenate is 3.9523mg prot/ml, calculate as follows:

$$\text{SOD inhibition percentage} = \frac{(A_{\text{Contrast}} - A_{\text{Contrast blank}}) - (A_{\text{Sample}} - A_{\text{Sample blank}})}{(A_{\text{Contrast}} - A_{\text{Contrast blank}})} \times 100\%$$

$$= \frac{(0.3214 - 0.0765) - (0.2575 - 0.0791)}{(0.3214 - 0.0765)} \times 100\% = 27.15\%$$

$$\text{SOD activity (U/g tissue)} = \text{SOD inhibition percentage} \div 50\% \times \text{Reaction system dilution times} \left(\frac{0.24ml}{0.02ml}\right) \times \text{Sample dilution times before assay} \div \frac{\text{Sample weight (g)}}{\text{Added buffer volume (ml)}}$$

$$= 27.15\% \div 50\% \times \frac{0.24ml}{0.02ml} \times 10 \div \frac{0.2g}{1.8ml} = 586.44 (\text{U/g tissue})$$

Whole Blood and Erythrocytes SOD Assay

Pretreatment:

Whole blood: Collect heparin anticoagulated whole blood, put upside down softly to mix sufficiently, take a certain amount of whole blood, add cold distilled water of 4 times volume, mix sufficiently by vortex, place quescently for 10 minutes in order to hemolyze completely (observe towards light, solution becomes limpid), it is considered as 5 times diluted hemolysate, then dilute hemolysate with distilled water to different concentrations for experiment.

Erythrocytes: Collect heparin anticoagulated whole blood, put upside down softly to mix sufficiently, remove upper layer of plasma after centrifuge, please control centrifuging speed according to species.

Species	Proper centrifuging speed
Mouse	1000~1500rpm
Rat, Rabbit	2000~2500rpm
Human	2500~3000rpm

Note: Too high a centrifuging speed will cause erythroclasis, which leads to hemolysis in plasma.

Add physiological saline of 3 times volume to underlayer of erythrocytes, put upside down softly to mix sufficiently, centrifuging at 500rpm for 5 minutes, remove supernatant and keep sediment erythrocytes (wash erythrocytes), take a certain amount of erythrocytes, add cold distilled water of 9 times volume, mix sufficiently by vortex for 30 seconds, place quescently for 10 minutes in order to hemolyze completely (observe towards light, solution becomes limpid), it is considered as 10 times hemolysate, then dilute hemolysate with distilled water to different concentrations for experiment.

Note 1: You should measure hemoglobin concentration in both 5 times hemolysate and 10 times hemolysate for result calculation. (You can buy hemoglobin assay solution from us, serial No.: C021) Note 2: If you do preliminary experiment probing for hemolysate of different concentrations, then please make sample blank for each concentration; if you do measurements in batches, then you only need to make 1~2 sample blank wells for same concentration.

Formulas for samples of whole blood and erythrocytes: SOD inhibition percentage formula:

$$\text{SOD inhibition percentage} = \frac{(A_{\text{Contrast}} - A_{\text{Contrast blank}}) - (A_{\text{Sample}} - A_{\text{Sample blank}})}{(A_{\text{Contrast}} - A_{\text{Contrast blank}})} \times 100\%$$

SOD Activity Formula:

$$\text{SOD activity (U/mgHb)} = \text{SOD inhibition percentage} \div 50\% \times \text{Reaction system dilution times} \left(\frac{0.24ml}{0.02ml}\right) \div \text{Hemoglobin concentration in sample to assay (mgHb/ml)}$$

Example:

Take heparin anticoagulated mouse whole blood, make 10 times hemolysate according to erythrocytes pretreatment, dilute hemolysate 50 times with double distilled water, take 20ul. Run as per the Assay Protocol shown herein above. In results, Contrast is 0.3214, Contrast blank is 0.0765, Sample is 0.1803, Sample blank is 0.0801, hemoglobin concentration in 10 times hemolysate is 41.5501mgHb/ml, calculate as follows:

$$\text{SOD inhibition percentage} = \frac{(A_{\text{Contrast}} - A_{\text{Contrast blank}}) - (A_{\text{Sample}} - A_{\text{Sample blank}})}{(A_{\text{Contrast}} - A_{\text{Contrast blank}})} \times 100\%$$

$$= \frac{(0.3214 - 0.0765) - (0.1803 - 0.0801)}{(0.3214 - 0.0765)} \times 100\% = 59.09\%$$

$$\text{SOD activity (U/mgHb)} = \text{SOD inhibition percentage} \div 50\% \times \text{Reaction system dilution times} \left(\frac{0.24ml}{0.02ml}\right) \div \text{Hemoglobin concentration in sample to assay (mgHb/ml)}$$

$$= 59.09\% \div 50\% \times \frac{0.24ml}{0.02ml} \div (41.5501 \div 50) = 17.07(U/mgHb)$$

Appendix VI: SOD Standard Curve Preparation

Pretreatment:
Take SOD standards, and use volumetric flask to make 100 ug/ml standard stock solution. Then dilute stock solution with double distilled water to different concentrations (50, 40, 25, 20, 10, 5, 4, 2 U/ml) for experiment.

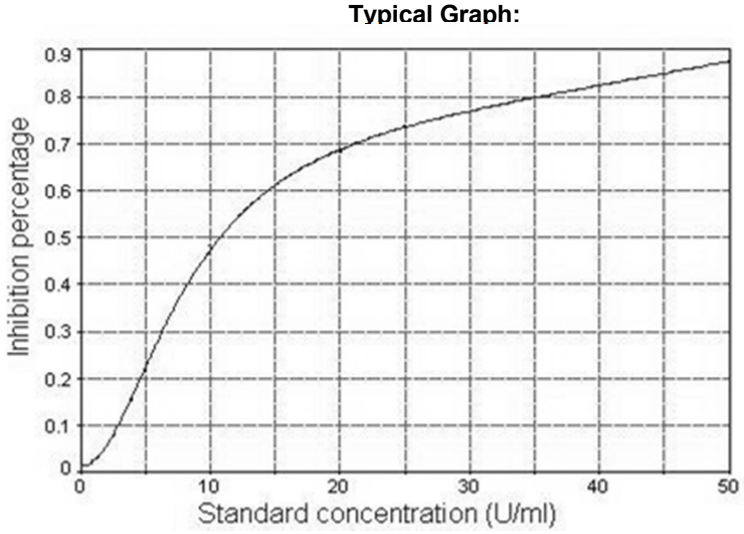
Calculation:

$$\text{SOD inhibition percentage} = \frac{(A_{\text{Contrast}} - A_{\text{Contrast blank}}) - (A_{\text{Sample}} - A_{\text{Sample blank}})}{(A_{\text{Contrast}} - A_{\text{Contrast blank}})} \times 100\%$$

Typical Results:

Well	Standard concentration	Absorbance	Absolute OD	Inhibition percentage
Contrast		0.3214		
Blank		0.0765		
1	2U/ml	0.3063	0.2298	6.16%
2	4 U/ml	0.2837	0.2072	15.38%
3	5 U/ml	0.2684	0.1919	21.63%
4	10 U/ml	0.2036	0.1271	48.12%
5	20 U/ml	0.1544	0.0779	68.18%
6	25 U/ml	0.1414	0.0649	73.48%
7	40 U/ml	0.1194	0.0429	82.50%
8	50 U/ml	0.1073	0.0308	87.42%

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.

References:

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