ELK Biotechnology

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Superoxide Dismutase WST-1 Assay Kit

（WST-1 method）

Serial No. BC009 Pack: 96T/48T

1.Reagent Conposition & Preparation

	Composition	Pack :96T	Pack : 48T	Storage
Reagent 1	Buffer	30ml×1 bottle	15ml×1 bottle	At 2～8℃ for 6 months
Reagent 2	Substrate stock solution	0.15 ml×1 vial	0.07 ml×1 vial	At 2～8℃ for 6 months
Substrate working solution preparation: Dilute substrate stock solution with buffer at ratio of 1 :200, mix sufficiently, this working solution should be used soon after preparation, consider solution volume according to you need. Residuary working solution can be stored at 2~8℃ for 7 days.				
Reagent 3	Enzyme stock solution	0.3ml×1 vial	0.15ml×1 vial	At -20℃ for 6 months
Reagent 4	Enzyme diluent	4ml×1 bottle	2ml×1 bottle	At 2～8℃ for 6 months
Enzyme working solution preparation: Dilute enzyme stock solution with enzyme diluent at ratio of 1:10, mix sufficiently, this working solution should be used soon after preparation, consider solution volume according to you need. Residuary working solution can be stored at 2~8℃ for 3 days.				

2.Required Equipments & Reagents

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

3. Operation Procedure

Well / Reagent	Contrast well	Contrast blank well	Sample well	Sample blank well
Sample	-	-	20μl	20μl
Double distilled water	20μl	20μl	-	-
Enzyme working solution	20μl		20μl	-
Enzyme diluent	-	20μl	-	20μl

Substrate working solution	200μl	200μl	200μl	200μl
Mix sufficiently, incubate at 37℃ for 20 minutes, use enzyme-labeled instrument to measure absorbances at 450nm.				

4. ANNOUNCEMENTS

(1) Please use multichannel micropipet to add substrate working solution, it is in order to /reduce operation time length, shorter time length leads to smaller errors between different wells.

(2) Use 96-wells microwell plate to operate, mix sufficiently to confirm complete contact between samples and reagents.

(3) Before you start formal assay, please choose 1~2 normal group samples, dilute to different concentrations to do pre-test. Choose the concentration of tube whose inhibition percentage is between 40%~60%, then start formal experiments in batches.

(4) This method has high specificity for SOD analysis, it removes disturbances of SOD analog chemicals. If you want to measure SOD analog chemicals, then you can buy SOD analogs assay kit from us.

(5) Inhibition percentage can reach 100% by using this method.

It only needs 1~2 contrast, 1~2 contrast blank and 1~2 sample blank wells for each batch of experiments.

5. 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, so their also have different formulas, you can find details in Appendix.

6. ASSAY SIGNIFICANCE

This kit can measure superoxide dismutase (SOD) activity in:

- Blood serum (or plasma),
- Cerebrospinal fluid,
- Hydrothorax and hydroperitoneum,
- Kidney dialysate, urine,
- Erythrocytes, leucocytes, blood platelets,
- Cardiac muscle cultured cells, tumor cultured cells,
- Various kinds of animal or plant tissue cells,
- Subcellsular level (mitochondria, microsome),
- Microbes,
- Medicines,
- Foods, drinks,
- Cosmetics.

This kit can be used for laboratory research only.

7. MAIN TECHNICAL PARAMETERS

- Contrast OD ≥ 0.2
- Reaction temperature: 37℃
- Wavelength = 450nm
- CV in batch = 5.05%
- CV between batches = 3.32%
- Detection limit: 0.5U/ml

8. ASSAY PRINCIPLE:

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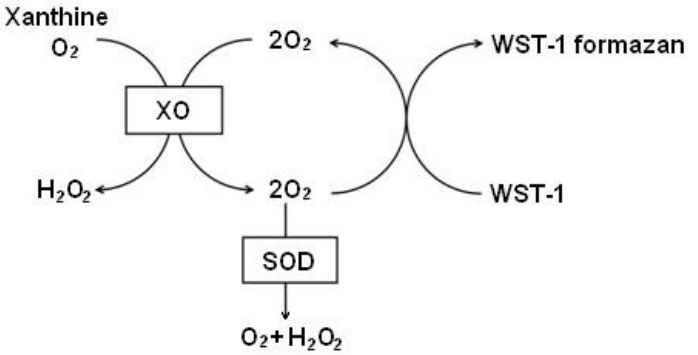

APPENDIX I: SOD assay in blood serum (or plasma) and other liquid samples

1. Sample pretreatment

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

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

2. Operation table

Reagent \ Well	Contrast well	Contrast blank well	Sample well	Sample blank well
Sample	-	-	20μl	20μl
Double distilled water	20μl	20μl	-	-
Enzyme working solution	20μl		20μl	-
Enzyme diluent	-	20μl	-	20μl
Substrate working solution	200μl	200μl	200μl	200μl
Mix sufficiently, incubate at 37℃ for 20 minutes, use enzyme-labeled instrument to measure absorbances at 450nm.				

It only needs 1~2 contrast, 1~2 contrast blank and 1~2 sample blank wells for each batch of experiments.

3. Calculation:

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

(2) Formula for SOD activity in blood serum (or plasma), cell culture supernatant, fruit juice, etc.

① SOD inhibition percentage calculation:

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

② SOD activity calculation:

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

4. Example:

Take 20μl normal human blood plasma sample (already 5 times diluted with physiological saline), operate according to operation table. In results, $A_{Contrast}$ is 0.3214, $A_{Contrast\ blank}$ is 0.0765, A_{Sample} is 0.2418, $A_{Sample\ blank}$ is 0.1031, calculate as follows:

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

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

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

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

APPENDIX II: Animal tissue sample SOD assay

1.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.

2. Operation table:

Reagent \ Well	Contrast well	Contrast blank well	Sample well	Sample blank well
Sample	-	-	20μl	20μl
Double distilled water	20μl	20μl	-	-
Enzyme working solution	20μl		20μl	-
Enzyme diluent	-	20μl	-	20μl
Substrate working solution	200μl	200μl	200μl	200μl
Mix sufficiently, incubate at 37℃ for 20 minutes, use enzyme-labeled instrument to measure absorbances at 450nm.				

It only needs 1~2 contrast, 1~2 contrast blank and 1~2 sample blank wells for each batch of experiments.

3. Calculation:

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

(2) Formula for animal tissue sample:

① SOD inhibition percentage calculation:

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

② SOD activity calculation:

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

4. Example:

Take 20μl 10% mouse brain tissue homogenate sample (already 100 times diluted with physiological saline), operate according to operation table. In results, $A_{Contrast}$ is 0.3214, $A_{Contrast\ blank}$ is 0.0765, A_{Sample} is 0.2047, $A_{Sample\ blank}$ is 0.0788, protein concentration in 1% brain homogenate is 0.435mgprot/ml, calculate as follows:

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

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

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

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

Appendix III: Cultured Cells SOD Assay

1. Pretreatment:

(1) Collection of cultured cells:

①. **Suspension cultured cells:** Can be collected by centrifugation directly (centrifugate at 1000 rpm for 10 minutes, remove supernatant and take sediment cells).

②. **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:

①. **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;

②. **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.

③. **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 can not be skipped.)

d. 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.

2. Operation table:

Add \ Well	Contrast well	Contrast blank well	Sample well	Sample blank well
Disrupted cell suspension	-	-	20μl	20μl
Double distilled water	20μl	20μl	-	-
Enzyme working solution	20μl		20μl	-
Enzyme diluent	-	20μl	-	20μl
Substrate working solution	200μl	200μl	200μl	200μl
Mix sufficiently, incubate at 37℃ for 20 minutes, use enzyme-labeled instrument to measure absorbances at 450nm.				

It only needs 1~2 contrast, 1~2 contrast blank and 1~2 sample blank wells for each batch of experiments.

3. Calculation:

(1) Definition: If SOD inhibition percentage reaches 50% in this reaction system, then corresponding enzyme quantity can be considered as 1 SOD activity unit (U).

(2) Formulas for culture cells sample.

① SOD inhibition percentage calculation:

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

② SOD activity calculation:

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

4. Example:

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

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

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

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

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

Appendix IV: Plant Tissue SOD Assay

1. 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.

2. Operation table:

Well / Add	Contrast tube	Contrast blank tube	Sample tube	Sample blank tube
Homogenate supernatant	-	-	20μl	20μl
Double distilled water	20μl	20μl	-	-
Enzyme working solution	20μl		20μl	-
Enzyme diluent	-	20μl	-	20μl
Substrate working solution	200μl	200μl	200μl	200μl
Mix sufficiently, incubate at 37℃ for 20 minutes, use enzyme-labeled instrument to measure absorbances at 450nm.				

It only needs 1~2 contrast, 1~2 contrast blank and 1~2 sample blank wells for each batch of experiments.

3. Calculation:

(1) Definition: If SOD inhibition percentage reaches 50% in this reaction system, then corresponding enzyme quantity can be considered as 1 SOD activity unit (U).

(2) Formulas for plant tissue samples:

① SOD inhibition percentage calculation:

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

② 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:

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

4. Examples:

(1) Take 0.2g pakchoi leaf tissue accurately, add 1.8 ml 0.1M phosphate buffer, cut tissue to small pieces, make 10% pakchoi leaf homogenate in icewater bath, dilute this homogenate 10 times by phosphate buffer, take 20μl diluted homogenate to operate according to the table. In results, A_{Contrast} is 0.3214, $A_{\text{Contrast blank}}$ is 0.0765, A_{Sample} is 0.2575, $A_{\text{Sample blank}}$ is 0.0791, protein concentration in 10% pakchoi leaf homogenate is 3.9523mgprot/ml, calculate as follows:

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

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

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

$$= 27.15\% \div 50\% \times \frac{0.24ml}{0.02ml} \div (3.9523 \div 10) = 16.49(U/mgprof)$$

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

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

② Take 0.2g chinese kale leaf tissue accurately, add 1.8 ml 0.1M phosphate buffer, cut tissue to small pieces, make 10% chinese kale leaf homogenate in icewater bath, dilute this homogenate 10 times by phosphate buffer, take 20μl diluted homogenate to operate according to the table. In results, A_{Contrast} is 0.3214, $A_{\text{Contrast blank}}$ is 0.0765, A_{Sample} is 0.2434, $A_{\text{Sample blank}}$ is 0.0811, protein concentration in 10% chinese kale leaf homogenate is 3.2641mgprot/ml, calculate as follows:

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

$$= \frac{(0.3214 - 0.0765) - (0.2434 - 0.0811)}{(0.3214 - 0.0765)} \times 100\% = 33.73\%$$

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

$$= 33.73\% \div 50\% \times \frac{0.24ml}{0.02ml} \div (3.2641 \div 10) = 24.80(U/mgprof)$$

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

$$= 33.73\% \div 50\% \times \frac{0.24ml}{0.02ml} \times 10 \div \frac{0.2g}{1.8ml} = 728.57(\text{U/g tissue})$$

Appendix V: Whole Blood and Erythrocytes SOD Assay

1. 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 centrifuging speed will cause erythroclasis, 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.

2. Operation table:

Well / Add	Constrast tube	Contrast blank tube	Sample tube	Sample blank tube
Hemolysate	-	-	20μl	20μl
Double distilled water	20μl	20μl	-	-
Enzyme working solution	20μl		20μl	-
Enzyme diluent	-	20μl	-	20μl
Substrate working solution	200μl	200μl	200μl	200μl
Mix sufficiently, incubate at 37℃ for 20 minutes, use enzyme-labeled instrument to measure absorbances at 450nm.				

It only needs 1~2 contrast, 1~2 contrast blank and 1~2 sample blank wells for each batch of experiments.

3. Calculation

(1) Definition: If SOD inhibition percentage reaches 50% in this reaction system, then corresponding enzyme quantity can be considered as 1 SOD activity unit (U).

(1) 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:

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

4. Example:

Take heparin anticoagulated mouse whole blood, make 10 times hemolysate according to erythrocytes pretreatment, dilute hemolysate 50 times with double distilled water, take 20μl to operatate according to operation table. In results, $A_{Contrast}$ is 0.3214, $A_{Contrast\ blank}$ is 0.0765, A_{Sample} is 0.1803, $A_{Sample\ blank}$ is 0.0801, hemoglobin concentration in 10 times hemolysate is 41.5501mgHb/ml, calculate as follows:

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

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

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

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

Appendix VI: SOD standard curve preparation

1. Pretreatment:

Take SOD standard (produced by Roche company), use volumetric flask to make 100μg/ml standard stock solution, then dilute stock solution with double distilled water to different concentrations (50, 40, 25, 20, 10, 5, 4, 2U/ml) for experiment.

2. Operation table:

Add \ Well	Constrast tube	Contrast blank tube	Sample tube
Hemolysate	-	-	20μl
Double distilled water	20μl	20μl	-
Enzyme working solution	20μl		20μl
Enzyme diluent	-	20μl	-
Substrate working solution	200μl	200μl	200μl
Mix sufficiently, incubate at 37℃ for 20 minutes, use enzyme-labeled instrument to measure absorbances at 450nm.			

3. Calculation:

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

4. 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%

5. Graph: