Technical Service Contact Information
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Hours: S-T 8:00 AM to 3:30 PM

TEB PAZHOUHAN RAZI

(TPR)

Superoxide Dismutase Activity Assay Kit (48/96 Tests)

Storage and Stability
This kit will perform as specified if **stored at 4°C**
Use before the **expiration date**

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About This Kit

Superoxide dismutases (SODs) play a major role in antioxidant defense regulations by catalyzing the dismutation of superoxide anion free radical (O_2^-) into molecular oxygen and hydrogen peroxide (H_2O_2). Therefore, the amount of enzyme in cellular and extracellular environment is crucial for the prevention of oxidative stress damages.

TPR's SOD Assay Kit is a well-established tool for measuring SOD in **serum, plasma, tissue, cell lysates and other biological fluids.** This kit measures SOD activity by utilizing tetrazolium salt, which produces a water-soluble formazan dye upon reduction with superoxide anion (Fig. 1). The formazan formation rate is inhibited by presence of SOD in environments and is measurable photometrically.

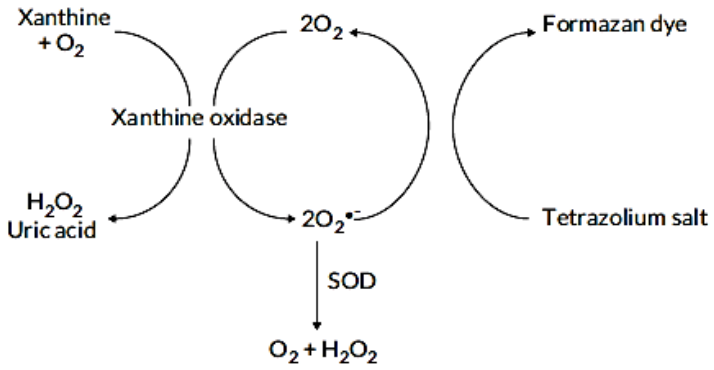

Fig. 1: Scheme of the SOD assay

Performance

- **Precision:** Human SOD with 8 replicates on 3 different days showed the intra and inter assay coefficient of variation 8.0% and 7.1% respectively.
- **Application:** This kit measures the activity of all isoforms of SOD enzyme and should work with all SODs (Cu/Zn, Mn and Fe SOD).

Components

Item Label	Item	48 Tests Kit	96 Tests Kit
Reagent 1 (R1)	Chromogenic Reagent	1 ml	1 ml (2 vials)
Reagent 2 (R2)	SOD Enzyme Solution	15 µl	30 µl
Reagent 3 (R3)	SOD Assay Buffer	20 ml	20 ml (2 vials)
Reagent 4 (R4)	Sample Dilution Buffer	12 ml	12 ml
-	96 Well Microplate	1 Plate	2 Plates
-	Technical Manual	1 Manual	1 Manual

Storage and stability

- This kit is stable for up to 6 months from date of receipt.
- Components are stable for 2 months after preparations, except Ready R2 Solution which is stable for 3 weeks only.

Required Materials Not Provided

- Distilled water
- Disposables tubes.
- PBS or 150 mM KCl (if using tissue samples)
- Dounce homogenizer (if using tissue)

Required Instrumentation

- Multiwell micro-plate reader (capable of measuring absorbance between 430-450 nm).
- Microfuge
- Incubator (37°C)
- 2-20 µl and 20-200 µl pipettes (multi-channel pipettes are preferred)

Preparation of Sample Solutions

- **Important Notes:**
  - ✓ All samples should be assayed immediately upon extraction or stored at -80°C for up to 1-2 months.
  - ✓ Always thaw samples on ice.

Cell Samples:

1. Harvest the amount of cells necessary for each assay (2 x 10^6 cells/ml recommended). For harvesting adherent cells, don't use proteolytic enzymes (like trypsin); rather use a rubber policeman.
2. Lyse cells by repeated cycles of freezing and thawing in ice cold PBS (pH 7.2-7.4).
3. Centrifuge at 14,000 x g for 5 minutes at +4°C.
4. Collect the supernatant and transfer to fresh tubes and place on ice.

Tissue Samples:

1. Weight out the amount of tissue necessary for each assay (10-100 mg wet tissue).
2. Perfuse or rinse the tissue with PBS or KCl (150 mM), to remove any red blood cells and clots.
3. Homogenize the tissue in 1 ml ice cold PBS or 150 mM KCl.
4. Centrifuge at 14,000 x g for 5 minutes at +4°C.
5. Collect the supernatant and transfer to fresh tubes and place on ice.

Note: RIPA buffer (without SDS) supplemented with PMSF protease inhibitors can also be used as lysis buffer.

Note: If it is desired to measure SOD activity from cytosol and mitochondria separately, tissue/cell samples can be prepared according to method described by *Mattiazzi et al (2002). JBC 277: 29626-33.*

Plasma and Blood Samples:

1. Collect whole blood using an anticoagulant such as heparin, EDTA, or citrate.
2. Centrifuge at 1,000 x g for 10 minutes at +4°C.
3. Carefully pipette off the plasma layer without disturbing the buffy layer, transfer to fresh tubes and place on ice.

Note: Plasma can be diluted 3-10 times with Sample Buffer prior to assay SOD activity.

4. Remove the buffy layer from the red cell pellet.
5. Re-suspend the erythrocytes in four times its volume of ice cold distilled water.
6. Centrifuge at 10,000 x g for 15 minutes at +4°C.
7. Collect the supernatant (erythrocyte lysate) and transfer to appropriate size tubes and place on ice.

Note: Red Blood Cell lysate can be diluted 100 times with Sample Buffer prior to assay SOD activity.

Serum Samples

1. Collect whole blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2000 x g for 15 minutes at 4°C and
4. Carefully pipette off the yellow serum layer without disturbing the buffy layer and transfer to new tubes and place on ice.

Note: Serum can be diluted 3-10 times with Sample Buffer prior to assay SOD activity.

Reagent Preparation

1) **Working Solution**: Mix 1 mL of R1 with 19 mL of R3. Aliquot working solution so that you have enough volume to perform the desired number of assays. Working solution is stable for 2 months if stored at 4°C in dark.

2) **R2 Ready Reagent:** Spin R2 Reagent for 30 seconds and mixed well with pipette before dilution. Dilute 15 µL of R2 with 600 µL of R4 (48 Tests Kit) / Dilute 30 µL of R2 with 1.2 ml of R4 (96 Tests kit). R2 Ready Reagent is stable up to 3 weeks at 4°C.

Things to Note

- For comparing SOD activities between different samples, these can be normalized on protein concentration or cell number. If necessary, the provided assay buffer can be used for diluting samples.
- All prepared reagents and samples must be equilibrated to room temperature just prior to use, and gently agitate.
- Assay all controls and samples in duplicate if possible.
- The SOD inhibition ratio can arrive 100%.
- Before the formal experiment, it needs to choose one or two samples for diluting a series of diluent and determine the dilution factor when the SOD inhibition ratio is 40%-60%.
- Since the superoxide will be released immediately after the addition of working solution, use a multiple channel pipette to avoid reaction time lag of each well.

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Assay Protocol

As described below, add samples and reagents in each well.

Blank 1 = 10 µL ddH2O
Blank 2 = 10µL sample
Blank 3 = 10 µL ddH2O
Sample wells = 10 µL sample

Component	Sample (µL)	Blank 1 (µL)	Blank 2 (µL)	Blank 3 (µL)
Sample solution	10	0	10	0
ddH2O	0	10	0	10
Working solution	200	200	200	200
R2 ready reagent	10	10	0	0
R4	0	0	10	10

1. Add 200 µL of Working Solution into each well.
2. Add 10 µL of R4 to Blank 2 and Blank 3.
3. Add 10 µL of R2 ready reagent to each sample well and Blank1.
4. Shake for 15 seconds and incubate at 37°C for 20 minutes.
5. Read the absorbance at 440-460 nm using a plate reader.

Calculations

Definition: when SOD inhibition ratio in this reaction system reaches 50%, the corresponding enzyme level is 1 SOD activity unit (U).

Formula for serum/plasma samples:

$$\text{Inhibition ratio of SOD (\%)} = \frac{(A_{blank1} - A_{blank3}) - (A_{sample} - A_{blank2})}{(A_{blank1} - A_{blank3})} \times 100$$

A = Absorbance

$$\text{SOD activity (U/mL)} = \text{Inhibition ratio of SOD (\%)} \div 50\% \times \left(\frac{220\ \mu l}{10\ \mu l}\right) \times \text{dilution factor of sample}$$

Formula for tissue and cells:

$$\text{Inhibition ratio of SOD (\%)} = \frac{(A_{blank1} - A_{blank3}) - (A_{sample} - A_{blank2})}{(A_{blank1} - A_{blank3})} \times 100$$

A = Absorbance

$$\text{SOD activity (U/mg prot)} = \text{Inhibition ratio of SOD (\%)} \div 50\% \times \left(\frac{220\ \mu l}{10\ \mu l}\right) \times \text{dilution factor of sample} \div \text{Protein concentration of sample (mg prot/mL)}$$

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References

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