Superoxide Dismutase (SOD) Colorimetric Activity Kit

Catalog Number EIASODC (192 tests)

Rev 1.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Superoxide Dismutase (SOD) Colorimetric Activity Kit is designed to measure all types of SOD activity (i.e., Cu/Zn, Mn, and Fe superoxide dismutases) in a variety of samples.

This assay measures the activity of SOD in serum, plasma, tissue lysates, cell lysates, and erythrocyte lysates. The assay was characterized with human SOD, but is expected to measure SOD activity in samples from other species.

Reactive oxygen species are produced during normal aerobic metabolism. These free radicals are removed or converted to other products *in vivo* by the activity of specific enzymes including a variety of superoxide dismutases which play important roles in scavenging O_2^-. Cu/Zn SOD (SOD1) is the major intracellular superoxide dismutase. MnSOD (SOD2) is the mitochondrial superoxide dismutase, and has similar properties to FeSOD found in some plants and bacteria. EC-SOD (SOD3) is the primary extracellular superoxide dismutase, and can be detected in serum, plasma, ascites, or synovial fluids.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity
Superoxide Dismutase Standard; 1 U/vial bovine superoxide dismutase erythrocyte, lyophilized	1 vial
Assay Buffer; containing detergents, stabilizers, and dye	50 mL
Clear 96-well Half Area Plate	2 plates
Substrate Concentrate (10X)	1.1 mL
Substrate Diluent; **keep tightly capped**	12 mL
Xanthine Oxidase Buffer; containing detergents and stabilizers	6 mL
Xanthine Oxidase Concentrate (25X)	225 µL

Materials required but not supplied

- Microtiter plate reader with software capable of measurement at or near 450 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- (*Optional*) 2 mM potassium cyanide

Procedural guidelines

Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Keep samples on ice to maintain enzyme activity.

Prepare samples

Sample	Procedure
Serum/ plasma	Collect samples • Collect blood in serum tubes. Centrifuge at 700 × *g* for 15 minutes at 4°C. Aspirate the supernatant. Assay immediately or freeze at –80°C. • Collect plasma in heparin or EDTA tubes. Centrifuge at 700 × *g* for 15 minutes at 4°C. Aspirate the pale yellow supernatant. Assay immediately or freeze at –80°C. **NOTE:** Some serum and plasma samples may contain significant hemoglobin concentrations and may require blanking prior to adding 1X Xanthine Oxidase (see Assay procedure, page 3).
Erythrocytes (RBCs)	1. Eyrthrocytes can be lysed by taking the pelleted RBCs from plasma as described in the previous section and adding 4 volumes of ice cold deionized water. 2. Centrifuge at 10,000 × *g* for 15 minutes at 4°C to remove debris. **NOTE:** Lysed RBCs will exhibit high background color and require blanking prior to adding 1X Xanthine Oxidase (see Assay procedure, page 3).

Prepare samples, continued

Sample	Procedure
Cell lysate	1. Prepare cell pellet. • For cells in suspension, centrifuge >1 × 10^6 cells at 250 x *g* for 10 minutes at 4°C, then discard the supernatant. • For adherent cells, rinse the culture dish with PBS, then harvest >1 × 10^6 cells by gentle trypsinization. Transfer to a tube on ice and centrifuge at 250 × *g* for 10 minutes at 4°C and discard the supernatant. 2. Resuspend the cell pellet in ice-cold PBS and transfer to a microcentrifuge tube on ice. Centrifuge the cells at 250 x *g* for 10 minutes at 4°C, discard the supernatant, and place the tube on ice. 3. Homogenize or sonicate the pellet in 0.5–1 mL of PBS per 100 mg of cells. Centrifuge at 1,500 × *g* for 10 minutes at 4°C. 4. Collect the supernatant and assay immediately, or store at -80°C. To measure cytosolic (SOD1, Cu/Zn) and/or mitochondrial SOD (SOD2, Mn) centrifuge supernatants at 10,000 × *g* for 15 minutes at 4°C. The supernatants will contain the cytosolic SOD and the cell pellets will contain mitochondrial SOD. To determine Mn SOD (SOD2) activity treat samples with 2 mM potassium cyanide. Addition of cyanide will inactivate other SOD enzymes
Tissue lysate	1. Wash tissue thoroughly with ice cold PBS prior to processing. 2. Homogenize tissue as described in previous section for cell lysate, steps 3 and 4.

Dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

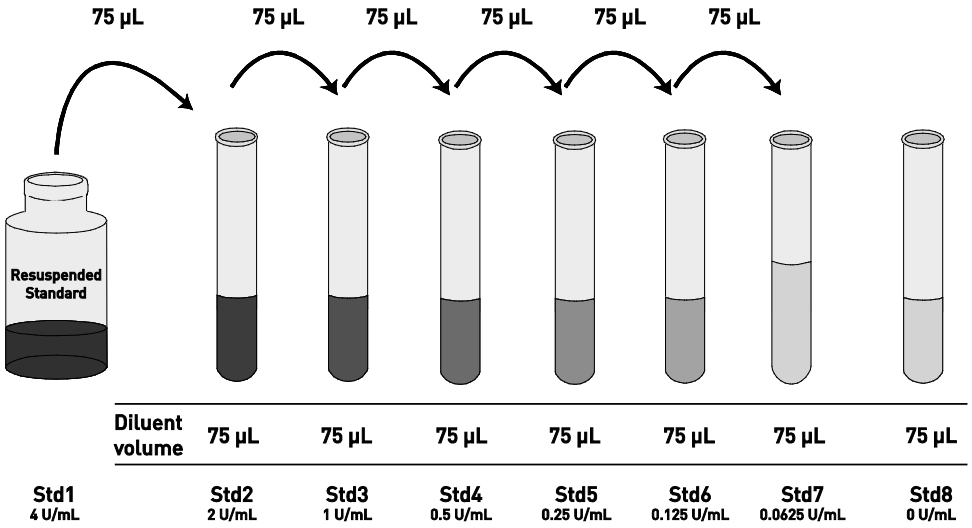
- Dilute **cell and tissue lysate** samples ≥1:4 in Assay Buffer.
- Dilute **serum and plasma** samples ≥1:5 in 1X Assay Buffer.
- Dilute **erythrocyte** samples ≥1:100 in Assay Buffer.
- Use all samples within **2 hours** of dilution.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: One unit of SOD is the amount of enzyme causing half the maximum inhibition of 1.5 mM nitro blue tetrazolium reduction in the presence of riboflavin at pH 7.8 and 25°C.

1. Reconstitute Superoxide Dismutase Standard by adding 250 µL Assay Buffer. Vortex and incubate for 5 minutes at room temperature. Label as 4 U/mL SOD.
2. Add 75 µL Assay Buffer to each of 7 tubes labeled as follows: 2, 1, 0.5, 0.25, 0.125, 0.0625, and 0 mU/mL SOD.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**. Store remaining reconstituted standard in frozen aliquots at –20°C or lower.



Prepare 1X Xanthine Oxidase

Vortex Xanthine Oxidase Concentrate prior to pipetting. Pipet from the bottom of the tube.

Reagent	½ plate	1 plate	1½ plates	2 plates
Xanthine Oxidase	50 µL	100 µL	150 µL	200 µL
Xanthine Oxidase Buffer	1.2 mL	2.4 mL	3.6 mL	4.8 mL
Total volume	1.25 mL	2.5 mL	3.75 mL	5 mL

Prepare 1X Substrate

Vortex the vial of Substrate Concentrate prior to pipetting.

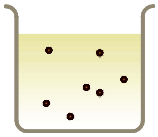
Note: Keep Substrate Diluent and 1X Substrate tightly capped when not in use.

Reagent	½ plate	1 plate	1½ plates	2 plates
Substrate Concentrate	250 µL	500 µL	750 µL	1 mL
Substrate Diluent	2.25 mL	4.5 mL	6.75 mL	9 mL
Total volume	2.5 mL	5 mL	7.5 mL	10 mL

Assay procedure

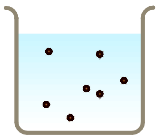
Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 20 minutes.**

IMPORTANT! Perform a standard curve with each assay.



Add sample and substrate

a. Add 10 µL of standards or diluted samples (see page 2) to the appropriate wells.
b. Add 50 µL of 1X Substrate into each well.



Add chromogenic detection reagent [1]

a. Add 25 µL of 1X Xanthine Oxidase into each well.
b. Incubate for 20 minutes at room temperature.

[1] Bright yellow colored samples can interfere with the high sensitivity format assay, and may require blanking prior to adding 1X Xanthine Oxidase.

To blank the plate, read the absorbance at 450 nm before adding the chromogenic detection reagent. Subtract the absorbance values from the blanking step from the corresponding absorbance measured for each sample.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the activity of unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals lower than that of the highest standard in Assay Buffer and reanalyze. Multiply the activity by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–4 U/mL SOD.

Standard SOD (U/mL)	Optical Density (450 nm)
4	0.075
2	0.110
1	0.169
0.5	0.262
0.25	0.359
0.125	0.480
0.0625	0.568
0	0.713

Intra-assay precision

Three samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	0.407	0.726	1.203
%CV	4.6	7.3	16.8

CV = Coefficient of Variation

Inter-assay precision

Three samples were assayed 16 times in duplicate by four operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	0.356	0.653	1.277
%CV	10.5	6.1	13.8

CV = Coefficient of Variation

Expected values

Five random adult human serum and plasma samples were diluted in Assay Buffer and run in the assay.

Sample	Range (U/mL)	Average (U/mL)
Serum	1.95–4.60	3.44
EDTA plasma [1]	2.24– 3.56	2.89

[1] Erythrocytes (RBCs) from these samples were normalized to hemoglobin (Hgb) levels using the Hemoglobin Colorimetric Detection Kit (Cat. No. EIAHGBC). The RBC activities ranged from 748 to 1,507 U/g Hgb with an average of 1052 U/g Hgb.

Linearity of dilution

Linearity was determined by assaying samples with high and low concentrations of SOD mixed in the ratios shown in the following table.

High Sample %	Low Sample %	Expected Conc. (U/mL)	Observed Conc. (U/mL)	% Recovery
80	20	0.987	0.920	93.2
60	40	0.789	0.776	98.4
40	60	0.590	0.534	90.4
20	80	0.392	0.414	105.6
			Mean Recovery	**96.9%**

Sensitivity

The analytical sensitivity of the assay is 0.044 U/mL SOD. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

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