Superoxide Dismutase Assay Kit

Reagent kit for the analysis of Superoxide Dismutase in cell extracts

Sufficient reagents for 100 Reactions

Catalog# 7500-100-K

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I. Background

The production of superoxide radicals by mitochondria is a substantial contributor to, if not the primary cause of pathology associated with neuro-degenerative diseases, ischemia reperfusion injury, atherosclerosis and aging.[1] Superoxide Dismutases (SOD) catalyze the dismutation of the superoxide radical ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2) and elemental oxygen (O_2) into the intermembrane space or mitochondrial matrix (Fig. 1), and thus provides an important defense against the toxicity of superoxide radicals.[2]

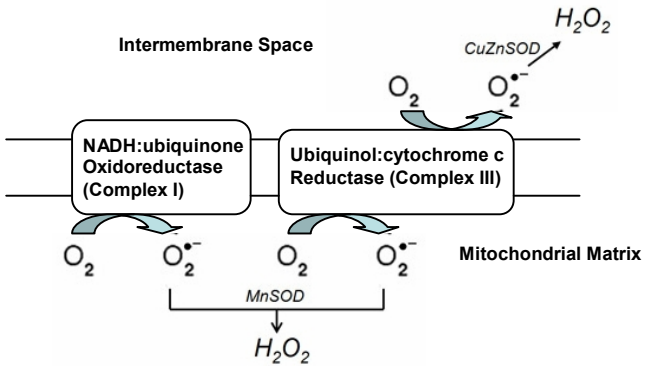


Figure 1. Hydrogen peroxide production by SODs.

Overexpression of SOD protects murine fibrosarcoma cells from apoptosis and promotes cell differentiation.[3] SOD also inhibits adriamycin-induced apoptosis in murine peritoneal macrophages.[4] Superoxide ions, generated from the conversion of xanthine to uric acid and hydrogen peroxide by xanthine oxidase (XOD), converts NBT to NBT-diformazan. NBT-diformazan absorbs light at 550 nm. SODs reduce superoxide ion concentrations and thereby lower the rate of NBT-diformazan formation. The extent of reduction in the appearance of NBT-diformazan is a measure of SOD activity present in your experimental sample (Fig. 2).

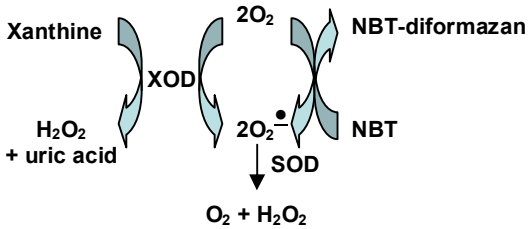


Figure 2. XOD and SOD cooperation in the inhibition of NBT-diformazan formation.

Trevigen's SOD assay is free of interference by other catalytic activities, and is ideal for assaying SOD in mammalian cell lysates. The kit contains the proper lysis buffer and the reagents needed for 100 experimental tests, 50 positive controls, and 50 negative controls. Unlike some other assay kits for SOD, this system is not greatly disturbed by trace metals. Each assay requires only about five minutes, and after a simple calculation, the percent inhibition of the formation of NBT-diformazan by SOD is converted to the relative activity of the sample.

II. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. The physical, chemical and toxicological properties of the provided products may not yet have been fully investigated, therefore, Trevigen recommends the use of gloves, lab coats, and eye protection while using these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products. MSDS are available on request.

III. Materials Supplied

Component	Quantity	Storage	Catalog #
SOD (1unit/µl)	200 µl	4 °C	7500-100-01
25X SOD Reaction Buffer	12.0 ml	4 °C	7500-100-02
Xanthine Solution	1.5 ml	4 °C	7500-100-03
NBT Solution	6.0 ml	4 °C	7500-100-04
XOD Solution	2.0 ml	4 °C	7500-100-05
20X Cell Lysis Solution	12.0 ml	4 °C	7500-100-06

IV. Reagents/Equipment Required But Not Supplied

Equipment

1. Visible spectrophotometer to read absorbance at 550 nm
2. Cuvettes (disposable or quartz, with at least a 1.5 ml volume)
3. Pipettor
4. Pipette tips
5, Pipette aid
6. Pasteur pipette and bulb
7. Centrifuge (for cell lysis)
8. Timer

Reagents

1. High quality, double-distilled H_2O

V. Reagent Preparation

Prior to each experiment, prepare the necessary amount of 1X Cell Lysis Solution by diluting the 20X Cell Lysis Solution with dH_2O. All other reagents are ready for use. Store all components at 4 °C until needed and avoid contamination.

VI. Assay Protocol

A. Cell Lysate Preparation

1. Detach adherent cells by gentle trypsinization. Count the cells and centrifuge at 250 x g for 10 minutes at 4 °C. Wash the cells once with cold 1 x PBS.
2. Suspend the pellet with 500 µl of Lysis Solution per 1-5 x 10^6 cells. Mix thoroughly by repeated pipetting. We recommend a sample volume of 400 µl.

3. Transfer the suspension to a 1.5 ml tube and centrifuge at 12-14,000 x g for 5 minutes at 4 °C. Place the supernatant into a clean 1.5 ml tube. Store on ice if you intend to assay for SOD immediately, or freeze at -80 °C for future use.

B. Tissue Lysate Preparation

1. Liver and other tissues may be lysed and processed in isotonic buffer (10mM Tris-Cl (pH 7.4), 200 mM mannitol, 50 mM sucrose, 1 mM EDTA) as described (7).

C. SOD Assay Procedure

1. The assay is performed at room temperature. All components, except cell lysates, should be brought to room temperature before use. The total reaction volume is 1.5 ml. The volume of the reagent components is 107.5 µl. Therefore, the volume of deionized water required is: 1500 µl – 107.5 µl – vol. sample. Briefly vortex each reagent immediately before use.

2. To a disposable cuvette add the following components in order:

dH_20	From Step C-1
25 x Reaction Buffer	60 µl
Xanthine Solution	7.5 µl

3. Mix thoroughly by repeated pipetting with a clean Pasteur pipette.

4. Add 30 µl of NBT Solution and repeat Step C-3.

5. Add your cell lysate and repeat Step C-3.

6. Place the cuvette into a spectrophotometer, read absorbance at 550 nm or set the absorbance reading to zero.

7. Just before use, briefly vortex the Xanthine Oxidase (XOD) Solution and add 10 µl to the cuvette. Quickly repeat Step C-3.

8. Immediately place the cuvette in the spectrophotometer, start a timer or stopwatch and record the absorbance reading every 30 seconds for 5 minutes. The first time point will be at 30 seconds and the final time point will be at 5 minutes 30 seconds.

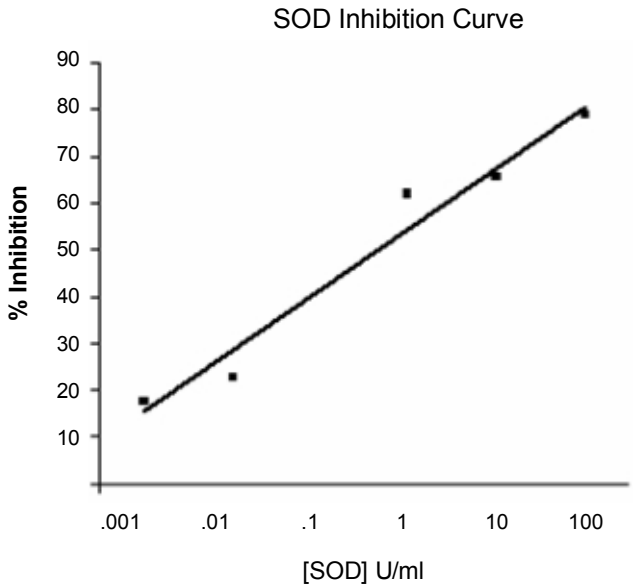
D. Controls

1. Negative Control
   A negative control must be performed. It includes all components except SOD or cell lysate. In this case, the increase in absorbance due to generation of superoxide radical proceeds maximally.

2. Positive Control
   The kit contains sufficient SOD for generating 10 standard curves or 50 individual positive controls. The SOD has an activity of 1 unit/µl (1 unit is the amount of SOD which inhibits the rate of increase in absorbance due to NBTdiformazan formation by 50%). A typical standard curve would include the following SOD concentrations: 0.01 unit, 0.1 unit, 1 unit, and 10 units. For the 0.01 and 0.1 unit points, dilute 1 µl of SOD to 100 µl and 10 µl, respectively,

with 1 x Reaction Buffer and use 1 µl of each. Add 1 µl and 10 µl of undiluted SOD for the 1 unit and 10 unit activity points, respectively. Briefly vortex the SOD immediately before use.

Figure 3: Plot of SOD concentration vs. % inhibition of the rate of increase of absorbance at 550nm due to the reduction of NBT to NBT-diformazan by the superoxide radical ($O_2^{\bullet-}$).



VII. Data Interpretation and SOD Activity Determination

1. Determine the rate of increase in absorbance units (A) per minute for the negative control and for the test sample(s).

$$\frac{A_{550}\text{ @ 5min.30 sec.} - A_{550}\text{ @ 30 sec.}}{5\text{ min}} = \Delta A_{550n}/\text{minute}$$

2. Determine the % inhibition for the test sample(s):

$$\frac{[(\Delta A_{550n}/\text{minute})_{\text{negative control}} - (\Delta A_{550n}/\text{minute})_{\text{test}}]}{(\Delta A_{550n}/\text{minute})_{\text{negative control}}} \times 100 = \%\ \text{Inhibition}$$

VIII. References

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IX. Related Products Available From Trevigen

Contact Trevigen for details of our unique product line for studying DNA damage and repair. All of Trevigen's kits include highly qualified enzymes, substrates, buffers, full instructions for use, and a synopsis specific for your kit.

PARP Assay Kits:

Catalog #	Description	Size
4667-50-K	PARP Activity Assay Kit	50 tests
4677-096-K	HT Universal Colorimetric PARP Assay w/ Histone Coated Strip Wells	96 samples
4676-096-K	Universal Chemiluminescent PARP Assay w/Histone Coated Strip Wells	96 samples
4667-250-01	Recombinant Human PARP Enzyme	250 µl
4668-100-1	Recombinant Human PARP (High Specific Activity)	1000 Units

DNA Damage Antibodies:

Catalog #	Description	Size
4410-PC-100	Fen-1	100 µl
4411-PC-100	γ-H2AX	100 µl
2372-PC-050	p53 Ack317	50 µl
2370-PC-050	p53 Ack379	50 µl
2371-PC-050	p53 Ack382	50 µl
2381-PC-100	p53 total	100 µl
4350-MC-100	UVssDNA	100 µg
4431-MC-100	XPF	100 µg
4421-MC-100	XRCC1	100 µg
4354-MC-050	anti-8-oxo-dG	50 µl

CometAssay™ Kits:

Catalog #	Description	Size
4250-050-K	CometAssay™ Kit	50 samples
4251-050-K	CometAssay™ Silver Staining Kit	50 samples
4252-040-K	CometAssay™ Higher Throughput Kit	40 samples
4253-096-K	CometAssay™ Kit 96 Wells	96 samples

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FLARE™ Assay Kits:

Catalog #	Description	Damage Recognized	Size
4040-100-FK 4040-100-FM	Fpg Kit	8-oxoguanine, DNA containing formamidopyrimidine moieties	75 samples 100 samples
4045-01K-FK 4045-01K-FM	Endonuclease III Kit	Thymine Glycol, 5,6-dihydro-thymine, urea, 5-hydroxy-6-hydrothymine, 5,6-dihydro-uracil, alloxan, 5-hydroxy-6-hydrouracil, uracil glycol, 5-hydroxy-5-methylhydantoin, 5-hydroxycytosine,5-hydroxy-uracil, methyl-tartonylurea, thymine ring saturated or fragmentation product	75 samples 100 samples
4130-100-FK 4130-100-FM	hOGG1 Kit	8-oxoguanine, DNA containing formamidopyrimidine moieties	75 samples 100 samples
4055-100-FK 4055-100-FM	T4-PDG Kit	Cis-syn isomers of cyclo-butane pyrimidine dimers	75 samples 100 samples
4065-100-FK 4065-100-FM	cv-PDG Kit	Cis-syn and trans-syn isomers of cyclobutane pyrimidine dimers	75 samples 100 samples
4100-100-FK 4100-100-FM	UVDE Kit	Cyclobutane pyrimidine dimers, (6-4) photoproducts	75 samples 100 samples

Oxidative Damage Kits

Catalog #	Description	Size
7511-100-K	HT Glutathione Assay Kit	384 tests
7512-100-K	HT Glutathione Peroxidase Assay Kit	480 tests
7513-500-K	HT Glutathione Reductase Assay Kit	500 tests
7501-500-K	HT Superoxide Dismutase Assay Kit	500 tests

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

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