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TECHNICAL INFORMATION

Catalog Number: 190117
Superoxide dismutase

Molecular Weight: ~32,500 Daltons
CAS # : 9054-89-1
Synonyms: Superoxide; Superoxide oxidoreductase; E.C.1.15.1.1
Source: *Bovine erythrocytes*
Form: Pale blue freeze-dried powder
Physical Description: Lyophilized salt-free powder
Unit Definition: That amount of enzyme causing a 50% inhibition in the rate of reduction of cytochrome C under the conditions of assay.
Solubility: Dissolves readily at 5mg/ml in 0.05M potassium phosphate/0.1mM EDTA pH 7.8, to give a clear slightly blue solution.
Assay Method: The method is based on the ability of superoxide dismutase to inhibit the reduction of nitro-blue tetrazolium by superoxide. One unit is defined as that amount of enzyme causing half the maximum inhibition of NBT reduction. The reaction velocity will depend largely on somewhat variable assay conditions such as light intensity and reaction temperature. Calibration of the method in individual laboratories is recommended. [2]
Reagents:

– 0.067 M Potassium phosphate buffer, pH 7.8
– 0.1 M Ethylene diamine tetraacetic acid (EDTA) containing 0.3 mM sodium cyanide
– 0.12 mM Riboflavin (store cold in a dark bottle)
– 1.5 mM Nitroblue tetrazolium (NBT) (store cold)

Enzyme:

– Prepare stock solution at one mg/ml.

Procedure:
Pipette into a series of tubes:

EDTA/Cyanide	0.2 ml
NBT	0.1 ml
Enzyme	*
Phosphate Buffer	q.s. to 3.0 ml

Include several tubes with no enzyme as controls
* A series of samples ranging from 0.1-10 micrograms is recommended. A tube containing approximately 100 micrograms will generally produce maximum inhibition. Place the tubes in a light box providing uniform light intensity. (A foil-lined box approximately 4' long X 8" X 6" with an internally mounted 40 W fluorescent bulb has been used successfully). Incubate the tubes for 5-8 minutes to achieve a standard temperature. At zero time and at timed intervals add 0.05 ml riboflavin. Incubate all tubes in the light box for 12 minutes and at timed intervals read A560. Determine percent inhibition of NBT reduction. Plot percent inhibition versus amount of enzyme in test. Determine the amount of enzyme resulting in one half of maximum inhibition.
Calculation:
Units/mg = 1000 / ug enzyme resulting in ½ max. inhibition
Availability:
Available in sizes:
3Ku
15Ku
30Ku
75Ku
References:

– McCord, J.M. and Fridovich, *J.Biol.Chem.*, **v.1,** 6049 (1969)

– Winterbourn, et. al., *J. Lab. Clin. Med.*, **v. 85**, 337 (1975).
– *Merck Index,* **12th Ed.,** No. 9177