Amplite™ Colorimetric Superoxide Dismutase Assay Kit

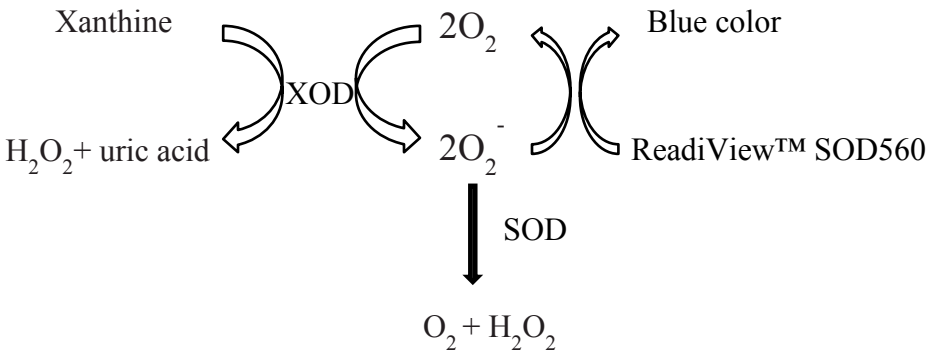
Blue Color

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 11305 (100 assays)	Keep at -20 °C and protect from light	Absorption microplate readers

Introduction

Superoxide dismutases (SOD) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. Superoxide is one of the main reactive oxygen species in cells. It is associated with neurodegenerative diseases, ischemia reperfusion injury, atherosclerosis and aging. SODs are an important antioxidant defense in nearly all cells exposed to superoxide radicals. It was reported that mice lacking SOD1 developed a wide range of pathologies, including hepatocellular carcinoma, an acceleration of age-related muscle mass loss, an earlier incidence of cataracts and a reduced lifespan. Overexpression of SOD protects murine fibrosarcoma cells from apoptosis and promotes cell differentiation.

The Amplite™ Colorimetric Superoxide Dismutases Assay Kit provides a quick and sensitive colorimetric method for measureing SOD. As shown belwo, xanthine is converted to superoxide radical ions ($O_2^{\cdot-}$), uric acid and hydrogen peroxide by xanthine oxidase (XO). Superoxide reacts with ReadiView™ SOD560 to generate a color product that absorbs around 560 nm. SOD competes the reaction of ReadiView™ SOD 560 with superoxide, thus reduce the absorption at 560 nm. The reduction in the absoption of ReadiView™ SOD 560 at 560 nm is proportional to SOD activity. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step.



Kit Components

Components	Amount
Component A: ReadiView™ SOD560	2 vials
Component B: 50X Xanthine	1 vial (100 µL)
Component C: Xanthine Oxidase	2 vials
Component D: SOD Standard	1 vial (500 Units)
Component E: Assay Buffer	20 mL

Assay Protocol for One 96-Well Plate

Brief Summary

SOD standards or test samples (50 µL) → Add SOD Assay Mixture (25 µL) → Add Enzyme reaction mixture (25 µL) → incubate at room temperature for 10-30 min → Read absorbance at 560 nm

Note: Thaw all the kit components to room temperature before starting the experiment.

1. **Prepare serial SOD (0 to 100 U/mL) standard solutions:**
   1.1 Add 50 µL Assay Buffer (Component E) into the vial of SOD Standard (Component D) to make 10 kU/mL SOD stock solution.
   Note: The unused SOD solution should be divided as single use aliquots and stored at -20°C.

   1.2 Add 10 µL of 10 kU/mL SOD solution in 990 µL of Assay Buffer (Component E) to get 100 U/mL SOD solution. Take 100 µL of 100 U/mL SOD solution to perform 1:10 serial and then 1:3 dilutions to get 10, 3, 1, 0.3, 0.1, 0.03 U/mL SOD standards solutions.

   1.3 Add SOD standards and SOD -containing test samples into a 96-well white wall/clear bottom or clear microplate as described in Tables 1 and 2.

Table 1. Layout of SOD standards and test samples in a clear 96-well microplate:

BL	BL	TS	TS	….	….						
SOD1	SOD1	….	….	….	….						
SOD2	SOD2										
SOD3	SOD3										
SOD4	SOD4										
SOD5	SOD5										
SOD6	SOD6										
SOD7	SOD7										

Note: SOD= Superoxide Dismutase Standards, BL=Blank Control, TS=Test Samples.

Table 2. Reagent composition for each well:

SOD Standard	**Blank Control**	**Test Sample**
Serial dilutions* (50 µL)	Assay buffer (Component E): 50 µL	50 µL

Note: Add the serially diluted superoxide dismutase standards from 0.03 U/mL to 100 U/mL into wells from SOD1 to SOD7 in duplicate.

2. **Prepare SOD assay mixture 1:**
   2.1 Add 2.5 mL Assay Buffer (Component E) to the bottle of ReadiView™ SOD560 (Component A), mix them well.

   2.2 Add 50 µL of 50X Xanthine (Component B) into the bottle of Component A (from Step 2.1) to make SOD assay mixture.
   Note: The SOD assay mixture should be prepared just before the experiment, and kept from light. The assay mixture is not stable and the unused portion should be discarded.

3. **Prepare SOD assay mixture 2:**
   3.1 Add 50 µL Assay Buffer to the vial of Xanthine Oxidase (Component C), and transfer 50 µL Xanthine Oxidase stock solution into 2.5 mL Assay Buffer (Component E) to make SOD assay mixture 2, mix well.

4. **Run SOD assay:**
   4.1 Add 25 µL of SOD assay mixture 1 (from Step 2.2) into each well of the SOD standard, blank control, and test samples (see Step 1, Tables 1 and 2).

   4.2 Add 25 µL of SOD assay mixture 2 (from Step 3) into each well of the SOD standard, blank control, and test samples (from Step 4.1).
   Note: For 384-well plate, add 25 µL of sample, 12.5 µL of SOD assay mixture 1, and 12.5 µL of SOD assay mixture 2 into each well.

   4.3 Incubate at room temperature for 30-60 minutes.

   4.4 Monitor the absorbance intensity 550 to 560 nm

Data Analysis

The absorbance in blank wells (no SOD, with the assay buffer only) is used as a control. For each SOD dose, the absorbance is reported as the change of the observed absorbance intensity subtracted from that of a SOD-free control. The SOD standard curve is shown in Figure 1.

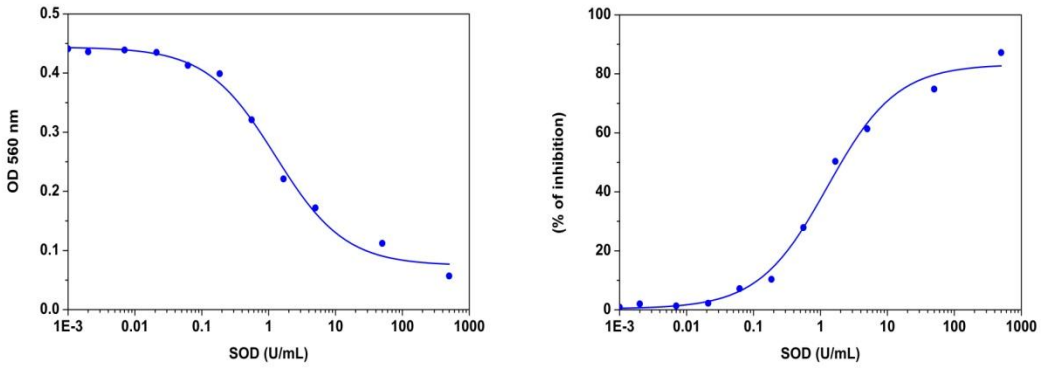


Figure 1. SOD dose response was measured with Amplite™ Colorimetric Superoxide Dismutase Assay Kit in a 96-well white wall/clear bottom plate with a Spectrum Max microplate reader (Molecular Devices). As low as 0.1 U/mL SOD was detected with 60 minutes incubation time (n=3).

References

1. Kussmaul L, Hirst J., The mechanism of superoxide production by NADH: ubiquinone oxidoreductase (complex I) from bovine heart mitochondria. *PNAS* 103: 7607-12, 2006.
2. Passos JF, Von Zglinicki T., Oxygen free radicals in cell senescence: are they signal transducers? *Free Radic Res* 40:1277-83, 2006.
3. Szeto HH., Mitochondria-targeted peptide antioxidants: novel neuroprotective agents. *AAPS J* 8:E521-31, 2006.
4. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. Biochem Pharmacol 37:837-41, 1988.
5. Nebot C, Moutet M, Huet P, Xu J-Z, Yadan J-C, Chaudiere J., Spectrophotometric assay of superoxide dismutase activity based on the activated auto-oxidation of a tetracyclic catechol. *Anal biochem* 214:442-51, 1993.