

EpiQuik™ Superoxide Dismutase Activity/Inhibition Assay Kit (Colorimetric)

Base Catalog # OP-0001

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ Superoxide Dismutase Activity/Inhibition Assay Kit (Colorimetric) is very suitable for quick measurement of SOD activity.

KIT CONTENTS

Components	96 assays OP-0001-1	2 × 96 assays OP-0001-2	5 × 96 assays OP-0001-3
SD1 (Dilution Buffer)	6 ml	12 ml	30 ml
SD2 (10X SOD Assay Buffer)	1 ml	2 ml	5 ml
SD3 (SOD Substrate)	1 ml	2 ml	5 ml
SD4 (SOD Indicator Solution)	1 ml	2 ml	5 ml
SD5 (Reaction Enzyme, 0.1U/ml)	1 ml	2 x 1 ml	5 ml
SD6 (SOD Standard, 10 U/μl)	50 μl	100 μl	200 μl
96-Well Microplate	1	2	5

SHIPPING & STORAGE

The kit is shipped in two parts: the first part at ambient room temperature and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store **SD5** at –20°C away from light. Avoid repeated freeze/thaw cycles; (2) Store **SD1** and **SD2** at room temperature; (3) Store all remaining components at 4°C away from light. All components of the kit are stable for 6 months from the date of shipment, when stored properly.

Note: Check if **SD2** contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.

GENERAL PRODUCT INFORMATION

Usage Limitation: The *EpiQuik*™ Superoxide Dismutase Activity/Inhibition Assay Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic application.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Intellectual Property: *EpiQuik*™ is a trademark of EpigenTek, Inc.

A BRIEF OVERVIEW

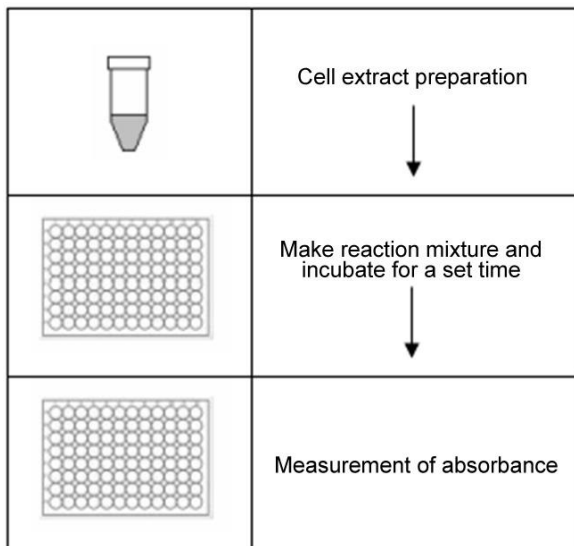
Superoxide dismutase (SOD) is one of the most important enzymes in defending oxidative stress. SOD catalyses the dismutation of superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen (O_2). Oxidative stress through reactive oxygen species (ROS) plays an important role in the etiology and progression in certain diseases such as cancer, heart disease, and various autoimmune disorders. ROS have shown to cause cellular damage, enzyme inactivation, and more particularly, epigenetic damage of DNA; thus, the determination of SOD activity is necessary in

various research fields related to human diseases. There are several methods used for measuring SOD activity, however, most of these methods have some drawbacks in selectivity, rapidity, and convenience. The *EpiQuik*[™] Superoxide Dismutase Activity/Inhibition Assay Kit (Colorimetric) addresses this problem by using a unique procedure to measure SOD activity. The kit has the following features:

- The fastest procedure available, which can be finished within 60 minutes.
- Very simple — just one step.
- High throughput microplate format with consistent reaction conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*[™] Superoxide Dismutase Activity/Inhibition Assay Kit (Colorimetric) utilizes a dye that produces a water-soluble formazan upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD. Therefore, the inhibition activity of SOD can be determined by measuring superoxide anion-caused formation of water-soluble dye (color intensity). The higher the SOD activity, the less the formation of formazan and the lower the OD value.



Schematic Procedure for Using the *EpiQuik*[™] Superoxide Dismutase Activity/Inhibition Assay Kit (Colorimetric)

PROTOCOL

1. Prepare cell or tissue extracts by using your own successful method. For your convenience and the best results, EpigenTek offers a cellular protein extraction kit (Cat. No. OP-0003) optimized for use in the *EpiQuik*[™] series.
2. Add the following solutions to each well: 50 μ l of **SD1**, 10 μ l of **SD2**, 10 μ l of **SD3**, 10 μ l of **SD4**, and 10 μ l of *cell extract*. Finally, add 10 μ l of **SD5**. Mix, cover plate with plate cover, and incubate

at 37°C. For the untreated control, add 10 μ l of **SD1** instead of cell extract. For the SOD positive control, dilute **SD6** with **SD1** at a 1:10 ratio, and add 10 μ l of **diluted SD6** instead of cell extract. For SOD inhibition, add 5 μ l of different amounts of tested inhibitors and reduce **SD1** volume to 45 μ l. For the blank, add 20 μ l of **SD1** instead of **SD5** and cell extract.

3. For kinetic studies, read sample OD 470 nm at different time intervals for a total of 60 minutes. For endpoint assay, incubate sample at 37°C for 45-60 minutes (brown color could be seen in the control), and then read sample OD 470 nm.
4. For simple calculation of SOD activity, measure the difference in absorbance intensity between "No inhibitor sample control" and "No extract sample control." For a more accurate calculation, the standard curve should be prepared.
5. To prepare the standard curve, add 50 μ l of **SD1**, 10 μ l of **SD2**, 10 μ l of **SD3**, 10 μ l of **SD4**, and 10 μ l of **SD6** at different amounts to each well (ex: 0.1-10 U). Finally, add 10 μ l of **SD5**. Mix, cover plate with plate cover, and incubate at 37°C for 30-45 minutes (if dose-response is not significant, extend time to 90-180 minutes).
6. Read sample OD 470 nm. Plot OD value versus amount of **SD6** and determine the slope as delta OD/Unit.
7. Calculate SOD activity using the following formula:

$$\text{Activity (Unit/min/ml)} = \frac{\text{OD (untreated control - blank)} - \text{OD (sample - blank)}}{(\text{slope} \times \text{incubation time})} \times \text{sample dilution}$$

Note: If the strip-well plate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate