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.

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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
User Guide	1	1	1

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.

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

A BRIEF OVERVIEW

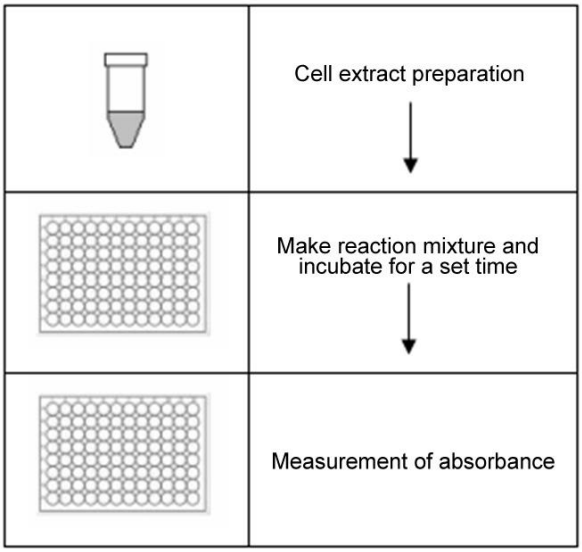
Superoxide dismutase (SOD) is one of the most important enzymes in defending oxidative stress. SOD catalyses the dismutation of superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen (O_2). Oxidative stress through reactive oxygen species (ROS) plays an important role in the etiology and 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 µl of **SD1**, 10 µl of **SD2**, 10 µl of **SD3**, 10 µl of **SD4**, and 10 µl of *cell extract*. Finally, add 10 µl of **SD5**. Mix, cover plate with plate cover, and incubate at 37°C. For the untreated control, add 10 µl of **SD1** instead of cell extract. For the SOD positive control, dilute **SD6** with **SD1** at a 1:10 ratio, and add 10 µl of **diluted SD6** instead of cell extract.

For SOD inhibition, add 5 µl of different amounts of tested inhibitors and reduce **SD1** volume to 45 µl. For the blank, add 20 µl 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 µl of **SD1**, 10 µl of **SD2**, 10 µl of **SD3**, 10 µl of **SD4**, and 10 µl of **SD6** at different amounts to each well (ex: 0.1-10 U). Finally, add 10 µl 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:

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