Superoxide Anion Content Assay Kit

(Sulfanilamide, Micro Method)

S1508310

Introduction:

The superoxide anion radical, a type of free radical produced during organism metabolism, can attack biological macromolecules such as lipids, proteins, nucleic acids, and polyunsaturated fatty acids, causing cross-linking or fragmentation, and damaging cellular structure and function. It is closely related to aging and pathological conditions, and research into scavenging superoxide anion radicals has garnered widespread attention. Molecular oxygen in organisms can undergo single-electron reduction to form the superoxide anion radical (O_2^-). O_2^- can directly act on macromolecules like proteins and nucleic acids, and can also generate reactive oxygen species such as hydroxyl radicals, singlet oxygen, hydrogen peroxide, and lipid peroxide radicals, which are damaging to cellular structure and function.

Detection Principle:

Under acidic conditions, two molecules of O_2^- react with hydroxylamine to produce one molecule of NO_2^-. NO_2^- then reacts with sulfanilic acid and naphthylamine to form a pink azo compound. The absorbance at 530 nm is measured using a microplate reader. Within a certain range, the color intensity is proportional to the O_2^- concentration. Based on the A_{530} value and the stoichiometric relationship between NO_2^- and O_2^- in the relevant reactions, the concentration of O_2^- in the sample can be calculated. This method is primarily used to determine the content or production rate of superoxide anion radicals in plant tissues.

Kit Contents and Storage Conditions:

S1508310	Component	100T	Storage
S1508310A	NO_2^- Standard (1 mM)	1 mL	2-8℃. Store in the dark.
S1508310B	O_2^- Lysis Buffer	250 mL	RT.
S1508310C	Hydroxylamine Solution	5 mL	2-8℃.
S1508310D	Sulfanilic Acid Color Development Solution	5 mL	2-8℃. Store in the dark.
S1508310E	Naphthylamine Color Development Solution	5 mL	2-8℃. Store in the dark.

Reagents, consumables and Equipments not provided:

- Microplate reader (capable of measuring absorbance at 530 nm).

- Mortar or homogenizer, Low-temperature centrifuge, Incubator or water bath.
- Centrifuge tubes or test tubes, 96-well plate.

Procedure:

1. Sample Preparation.

1.1 Plant Samples:

Take fresh plant tissue (normal or under stress), wash clean, dry, cut into small pieces, and quickly weigh 1–1.5 g. Add 2 mL of pre-chilled O_2^- Lysis Buffer and homogenize or grind on ice. Centrifuge at 4°C, 10,000 g for 10 minutes. The supernatant is the superoxide anion radical extract. Store at 4°C for use.

1.2 Plasma, Serum, and Urine Samples:

Plasma and serum prepared by standard methods can be directly used for assay with this kit. Store at 4°C for detection of superoxide anion radicals.

1.3 High-Activity Samples:

If the sample contains a high concentration of superoxide anion radicals, dilute appropriately with O_2^- Lysis Buffer.

2. Preparation of Series NO_2^- Standard Solutions:

Bring the NO_2^- Standard (1 mM) to room temperature, then continue dilution according to the table below:

Additive (μL)	Std.1	Std.2	Std.3	Std.4	Std.5	Std.6
NO_2^- Standard (1 mM)	1	2	3	4	5	6
Distilled Water	99	98	97	96	95	94
NO_2^- Content (μM)	10	20	30	40	50	60

3. O_2^- Sample Loading:

Set up blank, standard, and test wells according to the table below. Add solutions in the specified order, avoiding bubble formation. If the superoxide anion radical concentration in the sample is too high, reduce the sample volume or dilute appropriately before measurement. It is best to set up replicate wells for each sample.

Additive (μL)	Blank Well	Standard Well	Test Well
Distilled Water	100	-	-
Series NO_2^- Standards (No. 1–6)	-	100	-
Test Sample	-	-	25
O_2^- Lysis Buffer	-	-	25
Hydroxylamine Solution	-	-	50
Mix well and incubate in a 25°C water bath for 20 min.			
Sulfanilic Acid Color Development Solution	50	50	50
Naphthylamine Color Development Solution	50	50	50
Mix well and incubate in a 30°C water bath for 30 min.			

4. O_2^- Assay:

Zero the instrument with the blank. Measure the absorbance at 530 nm for the standard and test wells using a microplate reader (recorded as $A_{Standard}$ and A_{Test}).

5. Calculation of Results:

Plot a standard curve using the series NO_2^- standard contents (μM) from wells 1–6 as the x-axis and the corresponding absorbance as the y-axis. Calculate the NO_2^- content based on the absorbance of the test well. Calculate the superoxide anion radical (O_2^-) content in the specific sample using the following formulas:

O_2^- (μM/g) = 2 × n × V_T/ (W × V_S)

For serum, urine, and other liquid samples:

O_2^- (μM/mL) = 2 × n × N / V_S

Definition of Superoxide Anion Production Rate: The amount of superoxide anion produced per minute per gram of fresh tissue weight. Calculation formula:

Superoxide Anion Production Rate [μmol/(min·g)] = 2 × n × V_T / (W × t × V_S)

After measuring the protein content in the sample, the following formula can be used:

Superoxide Anion Production Rate [μmol/(min·mg)] = 2 × n × V_T (m × t × V_S)

Parameter Description:

2: Stoichiometric coefficient between NO_2^- and O_2^-.

n: NO_2^- content obtained from the standard curve (μM).

V_T: Total volume of superoxide anion radical extract (mL).

N: Sample dilution factor.

W: Sample fresh weight (g).

m: Protein content in the sample (mg).

t: Reaction time between sample and hydroxylamine (min) = 20.

V_S: Volume of extract added during measurement (mL).

Notes:

1. Experimental materials should be as fresh as possible. If not used immediately after collection, store at 4°C.
2. If the sample contains a high amount of chlorophyll, which may interfere with the assay, extract chlorophyll with an equal volume of ether or chloroform after incubation with hydroxylamine solution at 25°C for 20 minutes, then proceed with the color development reaction.
3. If a microplate reader is not available, a standard spectrophotometer can be used, but the minimum detection volume should be considered.
4. If the measured sample concentration is too high, dilute the sample with O_2^- Lysis Buffer and repeat the assay.
5. Sulfanilic Acid Color Development Solution and Naphthylamine Color Development Solution are highly irritating. Operate in a well-ventilated area and tighten bottle caps after use to avoid evaporation.

6. Use reagents as soon as possible after opening to avoid affecting subsequent experimental results.
7. This kit is intended for research use only and is not suitable for clinical diagnosis or other purposes.