Plant (mouse-ear cress) superoxide dismutase [Mn], mitochondrial (SODA) ELISA Kit

Catalog Number. CSB-EL022398PL

For the quantitative determination of endogenic plant (mouse-ear cress) superoxide dismutase [Mn], mitochondrial (SODA) concentrations in plant tissues.

This package insert must be read in its entirety before using this product.

If You Have Problems

Technical Service Contact information

Phone: 86-27-87582341
Fax: 86-27-87196150
Email: tech@cusabio.com
Web: www.cusabio.com

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

PRINCIPLE OF THE ASSAY

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with antigen. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific for SODA. The competitive inhibition reaction is launched between with pre-coated SODA and SODA in samples with the antibody. Then add a Horseradish Peroxidase (HRP) conjugated goat-anti-rabbit IgG antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of SODA in the sample. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

1.56 ng/ml-100 ng/ml.

SENSITIVITY

The minimum detectable dose of plant SODA is typically less than 0.39 ng/ml.
The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest plant SODA concentration that could be differentiated from zero. It was determined the mean O.D value of 20 replicates of the zero standard added by their three standard deviations.

PRECISION

Intra-assay Precision (Precision within an assay): CV%<10%
Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<20%
Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Reagents	Quantity
Assay plate	1(96 wells)
Standard (10 x concentrate)	1 x 200 µl
Antibody (100 x concentrate)	1 x 60 µl
HRP-conjugate(100 x concentrate)	1 x 120 µl
Antibody Diluent	1 x 10 ml
HRP-conjugate Diluent	1 x 20 ml
Sample Extraction Buffer (25 x concentrate)	1 x 20 ml
Sample Diluent	2 x 20 ml
Wash Buffer (25 x concentrate)	1 x 20 ml
TMB Substrate	1 x 10 ml
Stop Solution	1 x 10 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

STORAGE

Store at 2 - 8°C. Do not use past kit expiration date.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100 mL and 500 mL graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.
- Lyophilizer
- Stirrer

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

- **Xylem saps from plants** Xylem sap from wild plants can be obtained by cutting the plant about 10-15 cm above the ground (preferably early in the morning, to fully utilize the root pressure). Xylem sap collects in the silicon tube through root pressure. If there is risk of too much exposure to light, the tube should be wrapped in aluminum foil. Depending on the plant and the treatment, about 0.5mL should be obtained within 1-2 hours. The sap is collected from the silicon tube into an Eppendorf-vial, using a pipette, immediately frozen and stored for analysis at -80℃. This method has been used successfully on wheat, oil seed rape, maize and rice.
- **Crude extracts** Crude extracts of gingko, phoenix tree, rape *ect* have been tested to date with the extraction method describe below. Weigh out 0.5 g of freeze dried, finely ground material into a centrifuge tube containing 4.5 ml of sample extraction buffer. Shake the samples overnight in the cold (4-5℃) and dark. Spin down the solids and use the supernatant directly or diluted with buffer or H2O in the ELISA. For materials other than the ones mentioned above, the validity of this extraction method should be tested by both, cross-reaction test and confirming measurements with a HPLC - GC set-up.**(Dilution factor: 10)**

Note:

1. CUSABIO is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid loss of bioactivity and contamination.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
5. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.

REAGENT PREPARATION

Note:

- **Kindly use graduated containers to prepare the reagent.**
- Bring all reagents to room temperature (18-25°C) before use for 30min.
- Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- Making serial dilution in the wells directly is not permitted.
- To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10μl for once pipetting.
- Distilled water is recommended to be used to make the preparation for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.

- **Antibody (1x) -** Centrifuge the vial before opening.
  Antibody requires a 100-fold dilution. A suggested 100-fold dilution is 10µl of **Antibody** + 990 µl of **Antibody Diluent**.

- **HRP-conjugate (1x) -** Centrifuge the vial before opening.
  HRP-conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of **HRP- conjugate** + 990 µl of **HRP-conjugate Diluent**.

- **Sample Extraction Buffer(1x)-** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Sample Extraction Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Sample Extraction Buffer (1 x).

- **Wash Buffer(1x)-** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

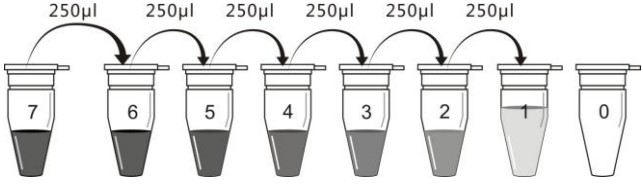
- **Standard**

  Centrifuge the standard vial at 6000-10000rpm for 30s before opening.

  Dilute the **Standard**(10x) with **Sample Diluent**. A suggested 10-fold dilution is 50 µl of **Standard**(10x) + 450 µl of **Sample Diluent**. This diluted **Standard (S7)** serves as the high standard (100 ng/ml).

  Do not substitute other diluents. Mix the standard to ensure complete dilution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

  Pipette 250 µl of **Sample Diluent** into each tube (S0-S6). Use the diluted **Standard** (S7) solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. **Sample Diluent** serves as the zero standard (0 ng/ml).



Tube	S7	S6	S5	S4	S3	S2	S1	S0
ng/ml	100	50	25	12.5	6.25	3.12	1.56	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a **Blank** well without any solution. Add 50µl of **Standard** or **Sample** per well. Standard need test in duplicate.
4. Add 50µl of **Antibody(1x)** to each well(not to Blank well). Mix well and then incubate for 30 minutes at 37°C.
5. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with **Wash Buffer** (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 10 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating ordecanting. Invert the plate and blot it against clean paper towels.
6. Add 100µl of **HRP-conjugate(1x)** to each well (not to Blank well). Mix well and then incubate for 30 minutes at 37°C.
7. Repeat the aspiration/wash process for five times as in step 5.
8. Add 90µl of **TMB Substrate** to each well, mix well. Incubate for 20 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
9. Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

6. TMB Substrate is easily contaminated. TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

CALCULATION OF RESULTS

Using the professional soft "Curve Expert 1.3" to make a standard curve is recommended, which can be downloaded from our web.

Average the duplicate readings for each standard and sample and subtract the average optical density of Blank.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the SODA concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.