Principle of the Plant NADH Dehydrogenase-J (ND-J) Assay Kit:

The Plant NADH Dehydrogenase-J (ND-J) assay kit is an enzyme-linked immunosorbent assay (ELISA) based on the solid-phase sandwich method. Standard samples with known concentrations and unknown samples are added to the microplate for detection. The test substance and biotin-labeled antibody are incubated simultaneously. After washing, avidin-labeled HRP is added. After another round of incubation and washing, unbound enzyme conjugate is removed, and substrate A, B, and the enzyme conjugate are added simultaneously. This generates color, and the intensity of the color is proportional to the concentration of the target substance in the sample.

Contents and Preparation of the Plant NADH Dehydrogenase-J (ND-J) Assay Kit:

Reagent components (to be stored at 2-8°C)	96-well plate configuration	48-well plate cover for partial use	
96/48 well ELISA plate	96 wells	48 wells	Ready to use
Plastic film plate cover	1 plate	1 plate	Ready to use
Standard samples	0.6ml	0.3ml	Dilute according to the instructions
Blank control	1.0ml	0.5ml	Ready to use
Standard sample dilution buffer	5ml	2.5ml	Ready to use
Biotinylated anti-TZA2 antibody labeled with a secondary enzyme	6ml	3.0ml	Ready to use
Affinity streptavidin-horseradish peroxidase (HRP)	10ml	5.0ml	Ready to use
Washing buffer	20ml	10ml	Dilute according to the instructions
Substrate A	6.0ml	3.0ml	Ready to use
Substrate B	6.0ml	3.0ml	Ready to use
Stop solution	6.0ml	3.0ml	Ready to use
Specimen dilution buffer	12ml	6.0ml	Ready to use

Materials Provided with the Plant NADH Dehydrogenase-J (ND-J) Assay Kit:

1) Distilled water.
2) Pipettors: 5 μl, 10 μl, 50 μl, 100 μl, 200 μl, 500 μl, 1000 μl.
3) Shaker and magnetic stirrer, etc.

Safety Precautions for the Plant NADH Dehydrogenase-J (ND-J) Assay Kit:

1) Avoid direct contact with stop solution, substrate A, and B. If contact occurs, rinse with water immediately.
2) Do not eat, drink, smoke, or use cosmetics during the experiment.
3) Do not aspirate any components of the assay kit with your mouth.

Operating Instructions for the Plant NADH Dehydrogenase-J (ND-J) Assay Kit:

1) Store the reagents as instructed on the label and allow them to equilibrate to room temperature before use. Discard any remaining diluted standard solutions.
2) Immediately return unused strips to the packaging bag and seal them to prevent deterioration.
3) Pack or cover any unused reagents. Do not mix reagents from different lots. Use before the expiration date.
4) Avoid cross-contamination by using disposable tips. When aspirating stop solution, substrate A, and B, avoid using pipettors with metal parts.
5) Prepare washing solution in clean plastic containers. Thoroughly mix all components and samples in the assay kit before use.
6) Fully tap dry the wash plate, avoiding direct contact of the absorbent paper with the reaction wells during water absorption.
7) Allow substrate A to evaporate and avoid leaving it open for an extended period. Substrate B is light-sensitive, so avoid prolonged exposure to light. Do not touch with hands as it is toxic. Read the OD value immediately after completing the experiment.
8) Follow a consistent order when adding reagents to ensure equal incubation time for all wells of the reaction plate.
9) Follow the instructions regarding incubation time, volume, and order of reagent addition.

Sample Collection, Processing, and Storage Methods for the Plant NADH Dehydrogenase-J (ND-J) Assay Kit:

1) Serum - Avoid any cell stimulation during the process. Use tubes that are free of pyrogens and endotoxins. After collecting blood, separate the serum and red blood cells quickly by centrifuging at 1000 × g for 10 minutes.
2) Plasma - EDTA, citrate, and heparin plasma can be used for testing. Remove particles by centrifuging at 1000 × g for 30 minutes.
3) Cell supernatant - Remove particles and polymers by centrifuging at 1000 × g for 10 minutes.
4) Tissue homogenate - Crush the tissue and add an appropriate amount of physiological saline. Centrifuge at 1000 × g for 10 minutes and collect the supernatant.
5) Storage - If the sample is not used immediately, divide it into small portions and store at -70℃ to avoid repeated freezing. Avoid using hemolyzed or hyperlipidemic blood as much as possible. If there are a large number of particles in the serum, centrifuge or filter before testing. Do not thaw at 37℃ or higher temperatures. Thaw at room temperature and ensure the sample is completely thawed.

Preparation of Reagents for the Plant NADH Dehydrogenase-J (ND-J) Assay Kit:

1) Standard solutions: Prepare the series dilution of standard solutions during the experiment and do not store them. Mix the standard solution well before dilution.
2) Dilution of Washing Buffer (50×): Dilute with distilled water at a ratio of 50 times.

Operating Steps for the Plant NADH Dehydrogenase-J (ND-J) Assay Kit:

1) Thoroughly mix all reagents before use. Avoid excessive foaming, as it may introduce air bubbles during sample addition and cause errors.
2) Determine the number of strips needed based on the quantity of test samples and the standard samples. It is recommended to use duplicate wells for each standard and blank. For each sample, use an appropriate number of wells, preferably in duplicate. Dilute the samples with sample diluent at a 1:1 ratio and add 50 µl to the reaction well.
3) Add 50 µl of diluted standard solution to the reaction wells and 50 µl of the test sample to the reaction wells. Immediately add 50 µl of biotin-labeled antibody. Cover the plate, gently shake to mix, and incubate at 37℃ for 1 hour.
4) Discard the liquid in the wells, fill each well with washing solution, shake for 30 seconds, discard the washing solution, and pat dry with absorbent paper. Repeat this process three times. If using a plate washer, increase the number of washes by one.
5) Add 80 µl of avidin-HRP to each well, gently shake to mix, and incubate at 37℃ for 30 minutes.
6) Discard the liquid in the wells, fill each well with washing solution, shake for 30 seconds, discard the washing solution, and pat dry with absorbent paper. Repeat this process three times. If using a plate washer, increase the number of washes by one.
7) Add 50 µl of substrate A and B to each well, gently shake to mix, and incubate at 37℃ for 10 minutes. Avoid exposure to light.
8) Remove the enzyme immunoassay plate and quickly add 50 µl of stop solution. Immediately measure the results after adding the stop solution.
9) Measure the OD value of each well at a wavelength of 450 nm.

Limitation of the Plant NADH Dehydrogenase-J (ND-J) Assay Kit:
Results above standard 6 are non-linear, and accurate results cannot be obtained based on this standard curve.

Performance of the Plant NADH Dehydrogenase-J (ND-J) Assay Kit:
1. Sensitivity: The minimum detection concentration is lower than that of standard 1. The dilution is linear. The correlation coefficient (R-value) between the linear regression of the sample and the expected concentration is 0.990.
2. Specificity: No reaction with other cellular factors.
3. Reproducibility: Both intra-plate and inter-plate coefficients of variation are less than 10%.

Interpretation and Analysis of Results from the Plant NADH Dehydrogenase-J (ND-J) Assay Kit:
1. Instrument readings: Read the OD values of each well at a wavelength of 450 nm using an enzyme-linked immunosorbent assay (ELISA) reader.
2. Plot a curve with the absorbance (OD value) on the y-axis and the corresponding concentration of the analyte standard on the x-axis. The content of the analyte in the sample can be calculated based on its OD value using the standard curve.