Principle of Plant 1,5-diphosphate ribulose bisphosphate carboxylase (rubisco) assay kit:

The Plant 1,5-diphosphate ribulose bisphosphate carboxylase (rubisco) assay kit is an enzyme-linked immunosorbent assay (ELISA) using the sandwich method. Known standard samples with known substance concentrations and unknown samples with unknown concentrations 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 conjugates are removed. Substrate A, B, and the enzyme conjugates are added simultaneously, resulting in a color change. The intensity of the color is proportional to the concentration of the test substance in the sample.

Contents and preparation of the Plant 1,5-diphosphate ribulose bisphosphate carboxylase (rubisco) 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 required for the Plant 1,5-diphosphate ribulose bisphosphate carboxylase (rubisco) assay kit:

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

Safety precautions for the Plant 1,5-diphosphate ribulose bisphosphate carboxylase (rubisco) assay kit:

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

Operating instructions for the Plant 1,5-diphosphate ribulose bisphosphate carboxylase (rubisco) assay kit:
1) Store the reagents as instructed on the labels and allow them to equilibrate to room temperature before use. Discard any partially used standard samples and do not save them.
2) Immediately return unused strips to their packaging and seal to prevent deterioration.
3) Properly package or cover any unused reagents. Do not mix reagents from different lots. Use before the expiration date.
4) Use disposable tips to avoid cross-contamination. Avoid using pipettes with metallic parts when aspirating stop solution and substrate A, B.
5) Prepare wash buffer in clean plastic containers. Thoroughly mix all components of the assay kit and samples before use.
6) When washing the microplate, tap dry completely. Do not place absorbent paper directly in the wells to absorb liquid.
7) Allow substrate A to evaporate and avoid keeping the lid open for a long time. Substrate B is light-sensitive and should be protected from prolonged exposure to light. Avoid contact with skin as it is toxic. Read OD values immediately after completing the experiment.
8) Add the reagents in a consistent order to ensure equal incubation time for all well rows.
9) Follow the instructions regarding incubation time, liquid volume, and sequence.

Methods for sample collection, processing, and storage for the Plant 1,5-diphosphate ribulose bisphosphate carboxylase (rubisco) assay kit:
1) Serum: Avoid any cell stimulation during the process. Use tubes that do not contain pyrogens and endotoxins. After collecting blood, quickly and carefully separate the serum from the red blood cells by centrifugation at 1000×g for 10 minutes.
2) Plasma: EDTA, citrate, and heparin plasma can be used for detection. Centrifuge at 1000×g for 30 minutes to remove particles.
3) Cell supernatant: Centrifuge at 1000×g for 10 minutes to remove particles and aggregates.
4) Tissue homogenate: Add the tissue to an appropriate amount of physiological saline and grind. Centrifuge at 1000×g for 10 minutes and collect the supernatant.
5) Storage: If samples are not used immediately, divide them into smaller portions and store at -70°C to avoid repeated freezing. Avoid using hemolyzed or hyperlipidemic blood. If there are a large number of particles in the serum, centrifuge or filter before testing. Do not heat or thaw at 37°C or higher temperatures. Thaw at room temperature and ensure thorough and even thawing.

Preparation of reagents for the Plant 1,5-diphosphate ribulose bisphosphate

carboxylase (rubisco) assay kit:

1) Standard samples: Dilute the standard samples according to the instructions before the experiment. They cannot be stored. Mix the standard samples well before dilution.
2) Dilution of wash buffer (50×): Dilute with distilled water at a 50-fold ratio.

Operating steps for the Plant 1,5-diphosphate ribulose bisphosphate carboxylase (rubisco) assay kit:

1) Before use, thoroughly mix all reagents. Avoid generating excessive foam to prevent the introduction of large air bubbles during pipetting, which may lead to errors.
2) Determine the number of strips required based on the quantity of test samples and the number of standard samples. It is recommended to have duplicate wells for each standard sample and blank wells. For each sample, use an appropriate number of wells and try to use duplicate wells whenever possible. Dilute the specimens 1:1 with specimen diluent and add 50μl to each well.
3) Add 50μl of diluted standard samples to the wells and 50μl of test samples to the wells. Immediately add 50μl of biotin-labeled antibody. Cover the plate with a membrane, gently shake to mix, and incubate at 37°C for 1 hour.
4) Discard the liquid from the wells, fill each well with wash buffer, shake for 30 seconds, discard the wash buffer, and pat dry with absorbent paper. Repeat this process three times. If using a microplate washer, increase the number of washes by one.
5) Add 80μl of avidin-HRP to each well, gently shake to mix, and incubate at 37°C for 30 minutes.
6) Discard the liquid from the wells, fill each well with wash buffer, shake for 30 seconds, discard the wash buffer, and pat dry with absorbent paper. Repeat this process three times. If using a microplate washer, increase the number of washes by one.
7) Add 50μl of substrate A and B to each well, gently shake to mix, and incubate at 37°C for 10 minutes. Avoid exposure to light.
8) Remove the microplate and quickly add 50μl of stop solution. Measure the results immediately after adding the stop solution.
9) Measure the OD values of each well at a wavelength of 450nm.

Limitations of the Plant 1,5-diphosphate ribulose bisphosphate carboxylase (rubisco) assay kit:

Results above standard 6 are non-linear, and accurate results cannot be obtained based on this standard curve.

Performance of the Plant 1,5-diphosphate ribulose bisphosphate carboxylase (rubisco) assay kit:

1. Sensitivity: The minimum detectable concentration is lower than that of standard 1. The dilution linearity is achieved. The coefficient of determination (R-value) for the linear regression of sample concentration against expected concentration is 0.990.
2. Specificity: No cross-reactivity with other cellular factors.
3. Reproducibility: Both within-plate and between-plate coefficients of variation are less than 10%.

Interpretation and analysis of the results of the Plant 1,5-diphosphate ribulose bisphosphate carboxylase (rubisco) assay kit:

1. Instrument readings: Read the OD values of each well on a microplate reader at a wavelength of 450 nm.
2. Plot a corresponding curve using the absorbance (OD value) as the y-axis and the corresponding concentrations of the test substance standard as the x-axis. The content of the test substance in the sample can be calculated from its OD value using the standard curve.