Product no: AS15 2994
Rubisco ELISA quantitation kit

Agrisera Antibodies

Product information

Background | This ELISA immunoassay utilize the principle of competitive inhibition for quantitative determination of endogenous Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) in plant tissues.

Rubisco antigen is pre-coated on the surface of the micro titer plate. Standards or samples are added to the plate wells including an antibody specific for Rubisco. The reaction of competitive inhibition is initiated between the pre-coated Rubisco and Rubisco in the samples with the antibody. A goat anti-rabbit IgG, HRP conjugated, antibody is added followed by a substrate solution and the color develops which is inversely proportional to the amount of Rubisco in the sample. The intensity of the color is measured after the reaction is stopped.

This Rubisco ELISA kit contains the following reagents:
Assay plate: 1x (96 wells)
Standards: 2 x 500 µl
Antibody: 1 x 60 µl (100 x concentrate)
HRP-conjugate: 1 x 120 µl (100 x concentrate)
Antibody Diluent: 1 x 10 ml
HRP-conjugate Diluent: 1 x 20 ml
Sample Diluent: 2 x 20 ml
Sample Extraction Buffer: 1 x 20 ml (25 x concentrate)
Washing Buffer: 1 x 20 ml (25 x concentrate)
TMB Substrate: 1 x 10 ml (store in the dark)
Stop Solution: 1 x 10 ml (contains acid, wear eye, hand, face and clothing protection)
Adhesive Strip: 4x (for 96 wells)

This kit is for research purpose only.

Storage: Store at 2-8°C. Opened kit can be stored at 2-8°C for maximum of 1 month.
Expiry date: Kit should not be used beyond expiry date.
Detection range: 3.12-800 µg/ml
Specificity/Sensitivity: High specificity and sensitivity for plant Rubisco. No significant cross reactivity has been observed.

Procedure instructions

PLEASE read CAREFULLY the whole manual before proceeding with your experiment

Before starting: As a recommendation, all samples and standards should be made in duplicates for this assay. Prepared samples should be stored at 2-8°C and used within 5 days, otherwise samples must be stored at -20°C or -80°C, for long time storage. Estimate sample concentration before the assay. Dilution of the samples can be necessary if the values are not in the range of the standard curve. To minimize imprecision caused by pipetting, use small volumes and ensure that your pipettes are calibrated.
Use sterile, distilled water when preparing the reagents and samples. For each step in the following procedure, the total handling time when adding reagents or samples to the assay plate should not be more than 10 min. Always use the plate sealers during the incubation steps. Do not allow wells to sit uncovered for extended periods between incubation steps either. Do not let the strips dry at any time during the assay.

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Sample preparations: Weigh 2 grams of fresh clean green leaf tissue, add 10ml of sample extraction Buffer (1x) and grind with mortar. Transfer the material to a micro centrifuge tube, incubate for 30 minutes at 4°C. After that, centrifuge at 5000 rpm for 10 minutes. Collect the supernatant for testing.
A five-fold dilution of the sample into sample diluent is recommended as a reference but optimal working dilution should be determined by the end user, appropriate for their experiment and sample. For some samples no dilution is necessary.

Manual

1. Place all reagents and samples at room temperature before use (at least for 30 min).

2. Centrifuge the samples after thawing.

3. Prepare the reagents, samples and standards

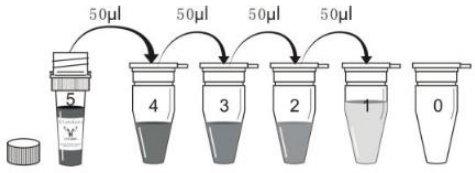
- Prepare the antibody sample (1x), a 100-fold dilution is required. Centrifuge the vial before opening. A 100-fold dilution is suggested, 10 µl of the Antibody in 990 µl of antibody diluent.

- Prepare the HRP-conjugate (1x), a 100-fold dilution is required. Centrifuge the vial before opening. A 100-fold dilution is suggested, 10 µl of the HRP- conjugate in 990 µl of HRP- conjugate diluent.

- Prepare the sample extraction buffer (1x). In case crystals have formed, warm the concentrate to room temperature and mix gently until the crystals have been dissolved. Dilute 20 ml of Sample Extraction Buffer. Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Sample Extraction Buffer (1x).

- Prepare the washing buffer (1x). In case crystals have formed, warm the concentrate to room temperature and mix gently until the crystals have been dissolved. Dilute 20 ml of Washing Buffer. Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

- Prepare the standards. Make fresh standards for each assay and use them within 4 hours. It is not recommended to make serial dilution directly into the wells. Centrifuge the standard vial at 6000-10000 rpm for 30s. Equilibrate the stock standard solution of 800 µg/ml for a minimum of 30 minutes to warm to room temperature prior to making dilutions. The standard itself is slightly red colored due to indicator. If sme precipitation occurs due to agglutination of the indicator, mix it well, centrifuge and use.
  Pipette 150 µl of Sample Diluent into each tube (S0-S4). Use the stock solution (S5) to produce a 4-fold dilution series. Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (800 µg/ml). Sample Diluent serves as blank (0 µg/ml).



Tube	S5	S4	S3	S2	S1	S0
µg/ml	800	200	50	12.5	3.12	0

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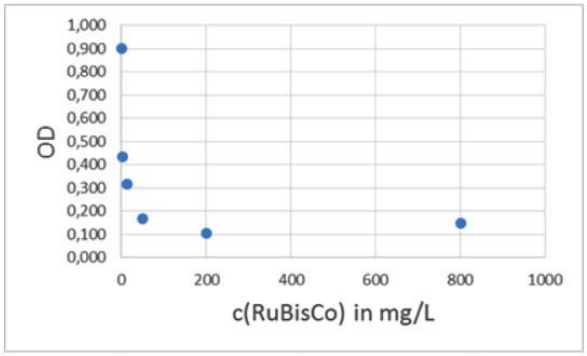

Sample	(450-570)nm - blank	c(RuBisCo) in mg/L
S0	0,9	0
S1	0,435	3,12
S2	0,317	12,5
S3	0,1665	50
S4	0,1065	200
S5	0,1485	800

Example of Rubisco standard curve. Note that developed color is inversely proportional to the amount of Rubisco in the sample.

4. Determine the number of wells to be used. For the remaining wells put them back into the pouch and seal the Ziploc. Unused wells should be stored at 4°C.

5. Set one blank well without any solution. Add 50 µl of standard or sample to each well. Add samples gently to avoid foaming and be careful not to touch the well walls.

6. Add 50 µl of Antibody (1x) to each well, except for the blank well. Mix well and incubate at 37°C for 60 min.

7. Wash the plate by filling the wells with washing buffer (200 µl in each well) using a squirt bottle, multi-channel pipette or auto-washer. Let it stand for 2 minutes, remove the liquid and repeat the washing process two times for a total of three washes. After the third wash remove all liquid and invert the plate against clean paper towels. Remove water and fingerprint on the bottom of the plate to avoid falsely reading results. The wash procedure is critical. Complete removal of liquid at each step is essential.

8. Add 100 µl of HRP-conjugate (1x) to each well (not to Blank well). Mix well and incubate at 37°C for 60 min.

9. Repeat the washing procedure for five times as in step 7.

10. Add 90 µl of TMB Substrate to each well and mix well. Incubate at 37°C for 20 minutes in the dark. (Check the color development from colorless to blue every 10 min, stop in advance if the color is too deep.)

11. Add 50 µl of Stop Solution to each well in the same order as the TMB substrate, mix thoroughly. The color will change from blue to yellow.

12. Read the optical density of each well within 5 minutes, at 450 nm. If wavelength correction should be available, set to 540 nm or 570 nm. Then subtract the readings at 540 nm or 570 nm from the

readings at 450 nm, to correct for optical deficiencies in the plate. Readings without correction, made at 450 nm can be higher and not as precise.

Results

Please use the "Curve Expert 1.3" for your calculations, which can be downloaded online. Average the duplicate readings and subtract the average optical density of the blank reading. The computer software capable of generating a four parameter logistic (4-PL) curve-fit can be used to reducing the data and make a standard curve. 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. To linearize the data, plot the log of the Rubisco concentrations against the log of the O.D. then use regression analysis to get the best fit line. The concentration read from the standard curve must be multiplied by the dilution factor if your samples have been diluted during the experiment.