Rubisco ELISA Kit (Plant) (OKGD00011)

Instructions for use

For the quantitative measurement of Rubisco in plant tissues.

Lot to lot variations may occur. Refer to the manual provided with the kit.

This product is intended for research use only.

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1. Background

Principle

Aviva Systems Biology Rubisco ELISA Kit (Plant) (OKGD00011) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for Rubisco has been pre-coated onto a 96-well plate. Standards or test samples are added to the wells, incubated and washed. A horseradish peroxidase (HRP) conjugated detector antibody specific for Rubisco is added, incubated and followed by washing. An enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by HRP generating a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm is quantitatively proportional to the amount of sample Rubisco captured in well.

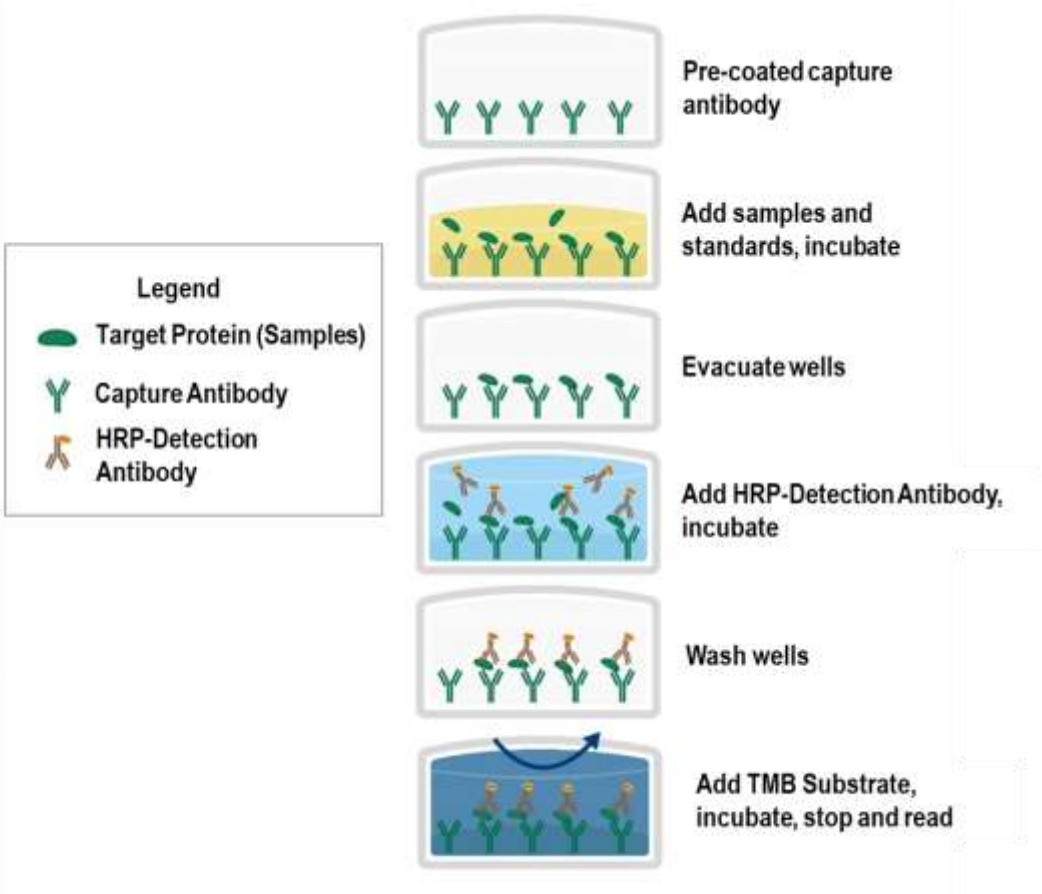
Target Background

Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (Rubisco) is an enzyme that is used in the Calvin Cycle to catalyze atmospheric carbon into more useable, high-energy molecules such as glucose or fructose in further plant processes. Rubisco catalyzes the carboxylation or oxygenation of ribulose-1,5-bisphosphate (RuBP) using carbon dioxide or oxygen in order to complete then regenerate the Calvin Cycle.[1]

General Specifications

General Specifications	
Range	13.7 - 10,000 ng/mL
Limit of Detection (LOD)	<0.086 ng/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Plant Rubisco UniProt ID: P08927 GeneID: 845212 Target Alias: Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase
Species Reactivity	Plant

2. Assay Summary



3. Storage and Stability

Upon receipt, store HRP Detection Antibody at -20°C, while other components of the kit can be stored at 4°C until expiration date (see Certificate of Analysis).

4. Kit Components

The following reagents are the provided contents of the kit:

Description	Quantity	Storage Conditions
Anti-Rubisco Microplate	96 wells (12 x 8 Well Strips)	HRP Detection Antibody:-20° C Other components: 4°C
Rubisco Lyophilized Standard	2 x 10,000 ng	
Rubisco HRP-Detection Antibody	1 x 30 µl	
Assay Diluent A	1 x 30 ml	
20X Wash Buffer	1 x 30 ml	
TMB Substrate	1 x 12 ml	
Stop Solution	1 x 12 ml	
Plate Seals	2	

5. Precautions

- Read instructions fully prior to beginning use of the assay kit.
- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit refer to the Material Safety Data Sheet (MSDS).
- Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

- Microplate reader capable of reading absorbance at 450 nm.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.
- 37°C Incubator (optional)

7. Technical Application Tips

- Do not mix or substitute components from other kits.
- To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
- Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
- Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
- Replicate wells are recommended for standards and samples.
- Cover microplate while incubating to prevent evaporation.
- Do not allow the microplate wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
- Completely remove of all liquids when washing to prevent cross contamination.
- Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
- For optimal results for inter- and intra-assay consistency, equilibrate all materials (except for standards) to 37°C prior to performing assay and perform all incubations at 37°C.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
- Samples that are hemolytic, lipemic, or contain precipitates, fibrin strands or bilirubin may cause inaccurate results due to interfering factors.
- TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

8. Reagent Preparation

- Equilibrate all materials to room temperature prior to use and prepare immediately prior to use.
- Prepare the Rubisco Standard no greater than 2 hours prior to performing experiment. A fresh lyophilized vial should be reconstituted for each experiment. Standards should be held on ice until use in the experiment.

8.1 Rubisco Standards

8.1.1 Prepare the **Rubisco Standard** no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.

8.1.2 Reconstitute one vial of the provided **10,000 ng Lyophilized Rubisco Standard** by adding 1 mL of **1x PBS** directly to the vial to achieve **Standard #1.** Close, and invert several times to dissolve. Do not vortex. Allow the vial to sit at room temperature for 10 minutes before use.

8.1.3 Prepare a set of serially diluted standards as follows:

8.1.3.1 Label tubes with the respective standard number.

8.1.3.2 Add the entire contents of the reconstituted standard to Tube #1**.**

8.1.3.3 Add 500 µL of **Assay Diluent** to the remaining tubes.

8.1.3.4 Prepare **Standard #2** by adding 250 µL of **Standard #1** from Tube #1 to Tube #2. Vortex briefly to mix.

8.1.3.5 Prepare **Standard #3** by adding 250 µL of **Standard #2** from Tube #2 to Tube #3. Vortex briefly to mix.

8.1.3.6 Prepare further serial dilutions in the remaining tubes. Reference the table below as a guide for serial dilution scheme.

8.1.3.7 The last tube is a blank standard (only **Assay Diluent**), which should be included with every experiment.

Standard Number (Tube)	Standard to Dilute (ng/mL)	Volume Standard to Dilute (µL)	Volume Assay Diluent (µL)	Total Volume (µL)	Final Concentration (ng/mL)
1	10,000 ng/mL Reconstituted Standard	1000	NA	1000	10,000 ng/mL
2	10,000 ng/mL	250	500	750	3333.3 ng/mL
3	3333.3 ng/mL	250	500	750	1111.1 ng/mL
4	1111.1 ng/mL	250	500	750	370.4 ng/mL
5	370.4 ng/mL	250	500	750	123.5 ng/mL
6	123.5 ng/mL	250	500	750	41.2 ng/mL
7	41.2 ng/mL	250	500	750	13.7 ng/mL
8	0 (Blank)	NA	500	500	0 (Blank)

8.2 Rubisco HRP-Detection Antibody

8.2.1 For each 96 wells to be used in the experiment prepare 11 mL of **Rubisco HRP-Detection Antibody solution** by following **Rubisco HRP- Detection Antibody dilution** protocol in COA.

8.2.2 Mix thoroughly and gently. Do not store at 1X concentration for future use.

8.3 1X Wash Buffer

8.3.1 If crystals have formed in the 20X Wash Buffer concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

8.3.2 Add the entire 30ml contents of the 20X Wash Buffer bottle to 570ml of ultra-pure water in a clean bottle.

8.3.3 Mix gently and thoroughly by magnetic stirrer.

8.3.4 Hold the Wash Buffer at room temperature until ready to use in the procedure. Store the prepared Wash Buffer at 4°C for no longer than 2 weeks. Do not freeze.

8.4 Microplate Preparation

8.4.1 Microplates are provided ready to use and do not require rinsing or blocking.

8.4.2 Any unused well strips should be sealed in the original packaging (including the desiccant) and stored at 4°C.

8.4.3 Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 General Sample Preparation and Storage

9.1.1 Add 2 g of fresh green leaf tissue to 10 mL of cold Extraction Buffer.

9.1.2 Homogenize by grinding with a mortar and pestle.

9.1.3 Transfer to a clean vial and hold at 4°C for 30 minutes.

9.1.4 Centrifuge the mixture at 5,000 rpm for 10 minutes.

9.1.5 Collect the supernatant for testing.

Store samples to be assayed at 2-8°C for 24 hours prior to being assayed.

For long term storage, aliquot and freeze samples at -20°C. Avoid repeated free-thaw cycles.

Samples not indicated in the manual must be tested to determine if the kit is valid.

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

10.1 Add 100 µL of the serially diluted **Rubisco Standards**, diluted samples or blank into wells of the **Anti-Rubisco Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.2 Cover the plate with plate sealer and incubate for 60 minutes at room temperature.

10.3 Remove the plate seal and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.5 Wash plate 5 times with **1X Wash Buffer** as follows:

10.5.1 Add 300 µL of **1X Wash Buffer** to each assay well.

10.5.2 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.5.3 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.5.4 Repeat steps 10.5.1 through 10.5.3 four more times.

10.6 Add 100 µL of prepared **Rubisco HRP-Detection Antibody** to each well.

10.7 Cover the plate with plate seal and incubate for 60 minutes at room temperature.

10.8 Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.9 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10 Wash plate **5 times** as in Step 10.5

10.11 Add 100 µL of **TMB Substrate** to each well and incubate **for 5-30 minutes in the dark.** Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.

(NOTE: TMB Substrate must be pre-warmed to room temperature before adding to the plate. **Incubation time must be determined by the user.** Optimal development can be visualized by blue shading in the top four standard wells. The blank (final standard point) should be faint blue to clear.)

10.12 Add 50 µL of **Stop Solution** to each well. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the **TMB Substrate.**

Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 10.12.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:

$$(\text{Relative } OD_{450}) = (\text{Well } OD_{450}) - (\text{Mean Blank Well } OD_{450})$$

The standard curve is generated by plotting the mean replicate **Relative OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The Rubisco concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software.

Note: If wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: If the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Reference

1. Andersson, I. and Backlund, A. 2008. Structure and function of Rubisco. Plant Physiol. and Biochem. 46(3):275-291

13. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

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