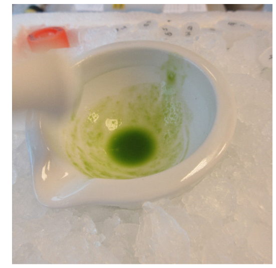
Jun 30, 2020

NADH-linked microtiter plate-based assay for measuring Rubisco activity & activation state – GAPDH-GlyPDH



In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.bgasjsee

Cristina Rodrigues Gabriel Sales[1], Anabela Silva[2], Elizabete Carmo-Silva[1]

[1]Lancaster Environment Centre, Lancaster University, Library Avenue, Lancaster, LA1 4YQ, UK;
[2]Biosystems & Integrative Sciences Institute (BioISI), Science Faculty of Lisbon University, Lisbon, 1749-016, Portugal

Cristina Rodrigues Gabriel Sales
Wild Bioscience

DOI: **https://dx.doi.org/10.17504/protocols.io.bgasjsee**

Protocol Citation: Cristina Rodrigues Gabriel Sales, Anabela Silva, Elizabete Carmo-Silva 2020. NADH-linked microtiter plate-based assay for measuring Rubisco activity & activation state – GAPDH-GlyPDH. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.bgasjsee**

Manuscript citation:
Sales CRG, Silva AB, Carmo-Silva E. 2020. Measuring Rubisco activity: challenges and opportunities of NADH-linked microtiter plate-based and 14C-based assays. Journal of Experimental Botany, https://doi.org/10.1093/jxb/eraa289

Protocol status: Working
We use this protocol and it's working

Created: May 12, 2020

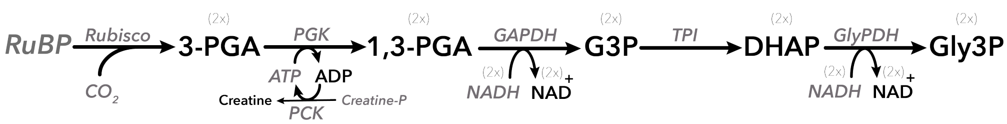
Last Modified: June 30, 2020

Protocol Integer ID: 36914

Keywords: Enzyme activity assay, Rubisco, Crop improvement , NADH-linked assay, GAPDH-GlyPDH, Plant phenotyping, Microtiter plate, measuring rubisco activity, rubisco activity, pga formation to nadh oxidation, reactions to couple rubp carboxylation, nadh oxidation, couple rubp carboxylation, glypdh this protocol, based assay, reaction,

Abstract

This protocol uses five reactions to couple RuBP carboxylation and 3-PGA formation to NADH oxidation to measure Rubisco activity, based on Kubien et al. (2011).



Note

During the NADH-linked assays, some Rubisco active sites might become carbamylated as the leaf extract is exposed to high CO_2 and Mg^{2+} in the assay buffer. Therefore, these assays are not suitable for measuring Rubisco initial activity and/or activation state at different levels in the canopy or in conditions in which low intercellular CO_2 is promoted (e.g., low light, drought stress, cold stress); the 30 second $^{14}CO_2$-based assay is recommended in such situations (protocol available in this collection).

Guidelines

1. Check the "Materials" tab for a list of all the chemicals used in this protocol.
2. In the "Steps" tab, there is a brief description of the materials and equipment necessary for the protocol execution.
3. In the "Steps" tab, there is information on preparation of solutions, procedures for determining Rubisco initial and total activities, and notes to take into consideration to ensure reliable results.
4. The references cited are at the end of the "Materials" tab.

Materials

MATERIALS

- Bicine **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #B3876
- Magnesium chloride hexahydrate ($MgCl2.6H2O$) **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #M2393
- Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #E1644
- Benzamidine **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #B6506
- ε-Aminocaproic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #A2504
- Sodium hydroxide (NaOH) **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #S5881
- 2-Mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #M6250
- DL-Dithiothreitol (DTT) **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #43819
- Phenylmethanesulfonyl fluoride (PMSF) **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #P7626
- Protease inhibitor cocktail **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #P9599
- D-Ribulose 1.5-bisphosphate sodium salt hydrate (RuBP) **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #83895
- Sodium bicarbonate ($NaHCO3$) **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #S6014
- β-Nicotinamide adenine dinucleotide reduced disodium salt hydrate (NADH) **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #N8129
- Phosphocreatine disodium salt hydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #P7936
- Adenosine 5′-triphosphate disodium salt hydrate (ATP) **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #A3377
- Creatine phosphokinase from rabbit muscle (PCK) **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #C3755
- Glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle (GAPDH) **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #G2267
- 3-Phosphoglyceric phosphokinase from bakers yeast (PGK) **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #P7634
- α-Glycerophosphate dehydrogenase/Triosephosphate isomerase (GlyPDH/TPI) **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #G1881
- Ethanol absolute 99.8 % **Fisher Scientific Catalog** #10437341

Citation

Kane HJ, Wilkin JM, Portis AR, Andrews TJ (1998)
. Potent inhibition of ribulose-bisphosphate carboxylase by an oxidized impurity in ribulose-1,5-bisphosphate.
Plant Physiology 117: 1059-1069.

https://doi.org/10.1104/pp.117.3.1059

LINK

Citation

Kubien DS, Brown CM, Kane HJ (2011). Quantifying the amount and activity of Rubisco in leaves.
Methods in Molecular Biology (Clifton, N.J.) 684: 349-362.

https://doi.org/10.1007/978-1-60761-925-3_27

LINK

Citation

Lampinen J, Raitio M, Perälä A, Oranen H, Harinen R (2015)
. Microplate based pathlength correction method for photometric DNA quantification assay.
Application Laboratory, Sample Preparation & Analysis, Thermo Fisher Scientific, Vantaa, Finland.

https://assets.thermofisher.com/TFS-Assets/LCD/Application-Notes/AN-SkanIT-Microplate-Based-Pathlength-Correction-Technical-Note

LINK

Citation

Sales CRG, Degen GE, Silva AB, Carmo-Silva E (2018)
. Spectrophotometric determination of Rubisco activity and activation state in leaf extracts.
In: Covshoff S, ed. Methods in Molecular Biology. New York: Humana Press 1770: 239-250.

https://doi.org/10.1007/978-1-4939-7786-4_14

LINK

Citation

Sharwood RE, Sonawane BV, Ghannoum O, Whitney SM (2016)
. Improved analysis of C4 and C3 photosynthesis via refined in vitro assays of their carbon fixation biochemistry.
Journal of Experimental Botany 67: 3137-3148.

https://doi.org/10.1093/jxb/erw154

LINK

Citation

Wong C-H (1980). Practical enzymatic syntheses of ribulose 1,5-bisphosphate and ribose 5-phosphate.
Journal of the American Chemical Society 102: 7938-7939.

https://doi.org/10.1021/ja00547a023

LINK

Troubleshooting

Safety warnings

Before using the protocol always check the Safety Data Sheet (SDS) for each chemical.

Before start

MATERIAL & EQUIPMENTS (for list of chemicals check "Materials" tab)

- Leaf sample frozen in -80°C
- Centrifuge for microtubes (speed 14000 *g*, 4 °C; VWR, Mega Star 600R)
- Microtiter plate reader (BMG Labtec, SpectroStarNano)
- 96-well microtiter plate with clear flat bottom (Thermo Scientific, 442404)
- Pipette set
- Mortar and pestle
- 1.5 mL microtubes

REAGENTS & SOLUTIONS

1 **REAGENTS & SOLUTIONS TO PREPARE BEFOREHAND**

Note

- Powder chemical stocks stored at -20°C: let warm up to room temperature on desiccant before opening container.
- Expensive chemicals purchased in very small amounts (mg), for which concentration in assay is not critical (e.g. in excess): trust quantity stated by the supplier and add ultrapure H_2O / solvent to container for final concentration (e.g. PCK).
- Protein and substrate solutions should typically be stored at -80°C.
- Freeze proteins in LN_2 before storing at -80°C. Store in small aliquots to prevent multiple freeze-thaw cycles. If using in consecutive days, protein solutions can be kept at 4°C.
- Buffers and solutions will last longer if filtered through 0.22 µm membrane.

1.1 **Basic extraction buffer (1x)**

[M] 50 millimolar (mM) Bicine-NaOH pH 8.2
[M] 20 millimolar (mM) $MgCl_2.6H_2O$
[M] 1 millimolar (mM) EDTA
[M] 2 millimolar (mM) Benzamidine
[M] 5 millimolar (mM) ε-Aminocaproic acid

- Dissolve in ultrapure H_2O; adjust pH to 8.2 with NaOH; degas the solution bubbling with nitrogen (5 min/10 mL), then add:
  [M] 50 millimolar (mM) 2-Mercaptoethanol
- Adjust for the final volume; it can be dispensed in aliquots (e.g. 50 mL Falcon tubes).
  -20 °C (storage)

1.2 [M] 1 Molarity (M) DTT

- Dissolve in ultrapure H_2O. 4 °C (storage)

1.3 [M] 100 millimolar (mM) PMSF

- Dissolve in ethanol 99%. 4 °C (storage)

1.4 Plant protease inhibitor cocktail

-20 °C (storage)

1.5 [M] 20 millimolar (mM) RuBP

-20 °C (storage)

Note

High purity RuBP (≥99%) is required to avoid interference in measurable activity due to the presence of RuBP-analogs that inhibit carboxylation (Kane et al., 1998; Sharwood et al., 2016). It is available commercially or it can be produced enzymatically from AMP-5’ monohydrate and ATP disodium salt (Wong, 1980).

2 **STOCK COMPONENTS FOR THE ASSAY BUFFER**

[M] 1 Molarity (M) Bicine-NaOH pH 8

- Dissolve in ultrapure H_2O; adjust pH to 8.2 with NaOH; filter through 0.22 µm membrane for long shelf life. 4 °C (storage)

[M] 1 Molarity (M) $MgCl_2.6H_2O$

- Dissolve in ultrapure H_2O; filter through 0.22 µm membrane for long shelf life. 4 °C (storage)

[M] 0.5 Molarity (M) NaHCO3

- Dissolve in ultrapure H_2O; filter through 0.22 µm membrane for long shelf life. 4 °C (storage)

[M] 1 Molarity (M) Phosphocreatine

Dissolve in ultrapure H_2O; aliquot; -80 °C (storage)

[M] 80 millimolar (mM) ATP

- Add powder to a tube, add some ultrapure H_2O to dissolve, add [M] 4 Molarity (M) NaOH (~10 µL/mL) to approximately pH 7 ; add ultrapure H_2O to the final volume; aliquot. -80 °C (storage)

Note

To test the pH use universal pH paper, add 1 µL of the solution and check colour.

[M] 14 millimolar (mM) NADH

- Dissolve in [M] 100 Molarity (M) Bicine-NaOH pH 8 ; aliquot. -80 °C (storage)

Note

Protect from light as it is light sensitive.
Please, check notes in section 2.1 to a more efficient way of aliquoting NADH.

[M] 1.8 KU/ml PCK

Dissolve all solid by adding [M] 100 Molarity (M) Bicine-NaOH pH 8 to the container; aliquot. -80 °C (storage)

[M] 5 KU/ml GAPDH

Dissolve all solid by adding [M] 100 Molarity (M) Bicine-NaOH pH 8 to the container; aliquot. -80 °C (storage)

[M] 17.9 KU/ml PGK

- Store at 4 °C when in ammonium sulfate suspension.
- Before use, it is necessary to buffer exchange as ammonium sulfate can affect the assay.
- Pipette a portion, e.g.,100 µL.
- Proceed to a buffer exchange using Amicon molecular weight cut-off of 10000 (Sigma-Aldrich); substitute ammonium sulfate for [M] 100 Molarity (M) Bicine-NaOH pH 8 in the same volume centrifuged (in this example 100 µL).
- Assay for TSP to know the concentration; aliquot. -80 °C (storage)

[M] 1.8 KU/ml GlyPDH + [M] 180 KU/ml TPI (GlyPDH/TPI)

- Store at 4 °C when in ammonium sulfate suspension.
- Before use, it is necessary to buffer exchange as ammonium sulfate can affect the assay.
- Pipette a portion, e.g.,100 µL.
- Proceed to a buffer exchange using Amicon molecular weight cut-off of 10000 (Sigma-Aldrich); substitute ammonium sulfate for [M] 100 Molarity (M) Bicine-NaOH pH 8 in the same volume centrifuged (in this example 100 µL).

- Assay for TSP to know the concentration; aliquot. -80 °C (storage)

2.1 **Complete assay buffer for Rubisco activity**

- The basic assay buffer (table below) can be prepared the day before the assays and kept at 4 °C , or prepared in advance (e.g. at the start of an experiment), snap-frozen in aliquots and kept at -80 °C .
- Each assay buffer aliquot should only be thawed once, as repeated freeze thawing can result in degradation of the coupling enzymes; thus, it is important to aliquot adequate volumes for use in a day.
- NADH is prepared separately, snap-frozen in aliquots and kept at -80 °C , and added to the assay buffer just before the assays.

Final concentration component	Stock	Volume in 200 µL assay (µL)
100 mM Bicine-NaOH pH 8.2	1 M	20
20 mM $MgCl_2$	1 M	4
10 mM $NaHCO_3$	0.5 M	4
5 mM DTT	1 M	1
5 mM Phosphocreatine	1 M	1
1 mM ATP	80 mM	2.5
25 U mL^{-1} PCK	1.8 KU mL^{-1}	2.8
25 U mL^{-1} GAPDH	5 KU mL^{-1}	1
25 U mL^{-1} PGK*	17.9 KU mL^{-1}	0.28
20 / 200 U mL^{-1} GlyPDH/TPI*	1.80 / 180 KU mL^{-1}	2.2
Total		38.8
To be prepared separately and added just before the assays		
0.4 mM NADH	14 mM	5.71
Total for each well		44.5

Note

*As PGK and GlyPDH/TPI need to be buffer exchanged, the stock concentration will change in each preparation, thus the volume added in 200 µL assay will change.

Note

We recommend using the same assay buffer for all the samples of the same experiment.

Note

Stock solutions and the assay buffer should thaw On ice. The assay buffer should be kept in a tube wrapped in aluminium foil On ice during the assays, as NADH is light sensitive.

3 **SOLUTIONS TO PREPARE JUST BEFORE USE**

- Prepared with reagents/solutions described in step 1.

3.1 **Complete extraction buffer**

1x Basic extraction buffer (from step 1.1)
[M] 10 millimolar (mM) DTT (from step 1.2)
[M] 1 millimolar (mM) PMSF (from step 1.3)
[M] 1 % (v/v) Plant protease inhibitor cocktail (from step 1.4)

- Prepare the volume considering the number of extractions to be performed throughout the day plus two extras (to have a little excess). Mix all together. On ice

Note

The volume of extraction buffer will depend on the size of the leaf sample and the protein content, therefore it is species dependent and should be tested beforehand. Rubisco concentration in the assays should be approx. 15 µg mL^{-1} for purified enzyme and between 10-40 µg mL^{-1} for non-purified enzyme. Rubisco amounts above these values may limit the sensitivity of the NADH-linked assays.

Note

To test if the assay is giving reliable results (i.e, none of the chemicals are limiting the reactions) it is important to always perform a test when the plant species and/or growth conditions change. Perform the assay with different extract concentration (e.g. 1/2 the amount, 1/5 of the amount, etc) and check if the activity expressed per protein content (TSP or Rubisco) is mantained.

3.2 **Complete assay buffer**

- Thaw On ice the assay buffer and NADH aliquots (prepared according to step 2.1) to be used in the day.
- Mix the correspondent volume of both solutions together. Keep On ice , wrapped in aluminium foil.

Note

Example of how to prepare the complete assay buffer: e.g. 20 samples in a day
- 20 samples x (1 Blank + 3xTotal + 3xInitial activity assays) = 140 wells
- Assay buffer without NADH per well = 38.8, for 140 wells = 38.8 x 140 = 5432µL
- NADH per well = 5.71, for 140 wells = 5.71 x 140 = 799.4 µL
- Mix both together (5432 + 799.4 µL)

PROCEDURE

4 **START**

- Thaw the frozen solutions that will be used in the day.
- Prepare CO_2-free ultrapure H_2O by bubbling with nitrogen (5 min/100 mL).
- Turn on the microplate reader and set up for the temperature that Rubisco activity will be performed, select kinetic protocol at 340 nm.

Note

The temperature to be used for the Rubisco activity measurement depends on the experiment goals. Typical measurement temperatures are 25 °C (standard) and 30 °C , depending on the species. Assays can be performed at a range of temperatures, however high temperatures might lead to evaporation of the assay mix and, since rates will be faster, the assay might become less sensitive.

- Turn on the centrifuge and set to 4 °C .
- Collect samples from -80 °C into liquid nitrogen container.
- Prepare the complete extraction buffer (from step 3.1) and the complete assay buffer (from step 3.2) and keep it On ice .

5 **EXTRACTIONS & RUBISCO ASSAYS**

5.1

- Before starting the extraction, pipette to the 96-well microtiter plate 150.5 µL CO_2-free ultrapure H_2O for the blank (singlet) and 144.5 µL for the samples into each well (6 wells, i.e., triplicates for initial activities and triplicates for total activities), followed by 44.5 µL of complete assay buffer (from step 3.2).
- Gently mix components by pipetting up and down 5 times whilst stirring. Add 6 µL of 20 mM RuBP (from step 1.5) to the wells for measuring initial Rubisco activity (see table below). Cover to protect from light. Proceed to extraction.

5.2 **Extraction**

- Add the complete extraction buffer (from step 3.1) to an ice-cold mortar.
- Take a sample from the liquid nitrogen container and add to the mortar.
- Grind the sample thoroughly for 00:00:30 to maximum of 00:01:00 .
- Collect the homogenate into an ice-cold 1.5 mL microtube and centrifuge 14000 x g, 4°C, 00:01:00 .

Note

To prevent Rubisco deactivation (or even denaturation) the extraction should not take more than 1 min and it should be done in a ice-cold mortar, keeping the sample cold at all times. In our hands, with the extraction buffer described (containing protease inhibitors, mercaptoethanol and DTT, which keeps the enzyme reduced) 1 min centrifugation does not impact Rubisco activity. However, this should be tested for each species and extraction buffer used.

- When centrifugation stops, take the extract supernatant into another ice-cold 1.5 mL microtube.
- Proceed to the Rubisco assays straight away.
- Add 5 µL of sample supernatant to the wells for total activity first, followed by those for initial activity, mixing well by pipetting up and down 10 times whilst stirring. Place microplate in the reader and start monitoring the change in absorbance at 340 nm immediately. The addition of the extract initiates the reaction for the initial activity assays, which is measured while incubating Rubisco with CO_2 and $MgCl_2$ in the absence of RuBP (total activity) for 00:03:00 at 30 °C to enable carbamylation of the enzyme. The absorbance value should start decreasing in the wells for the initial activity assay (containing RuBP).
- Pause the reading in order to add 6 µL of 20 mM RuBP (from step 1.5) to the wells for measurement of total Rubisco activity 00:03:00 after addition of sample supernatant.

- Place the microplate in the reader and continue monitoring the change in absorbance.
- The reading can be stopped once the reaction reaches a plateau.

Note

Considering that the protein concentration in the extract is as suggested in step 3.1, 5 µL of sample supernatant will give a good NADH consumption rate. The rate can be adjusted by adding more or less supernatant, but note that the amount of CO_2-free ultrapure H_2O added in the wells will change (as the final volume needs to be 200 µL).

Note

The Initial activity assays start with extract addition, while total activity assays start with addition of RuBP after 3 min of extract incubation with CO_2 and Mg^{2+} to allow for Rubisco carbamylation.

Note

This protocol can be adapted for measuring Rubisco activity with purified enzyme. In this case, Rubisco is frequently pre-activated and initial activity assays are performed.

- Below is a pipetting scheme for the microplate assay

	Volume to add (µL)		
Solution (in pipetting order)	**Blank**	**Initial activity**	**Total activity**
To be added before the extraction			
CO_2-free H_2O	150.5	144.5	144.5
Complete assay buffer	44.5	44.5	44.5
20 mM RuBP	0	6	0
To be proceeded after the extraction			
Leaf extract	5	5	5
Start measuring absorbance at 340 nm while incubating the plate at the desired temperature (e.g. 30°C). Pause reading after 3 min to start reaction for total activity.			
20 mM RuBP	0	0	6
Continue measuring absorbance at 340 nm until a plateau is reached.			

Note

Conducting measurements at 30°C provides fast rates and reliable slopes, but the temperature can be adjusted according to the experimental aims and plant species used.

Note

It is important to ensure that air bubbles are not introduced in the wells during the pipetting steps, as these will interfere with the absorbance measurements.

CALCULATIONS

6

- The activity of Rubisco is inferred from the consumption of RuBP (µmol s^{-1}) measured by absorbance change per second at 340 nm due to NADH oxidation, using an extinction coefficient of 6220 $M^{-1}cm^{-1}$ or 6.22 μmol^{-1} mL cm^{-1}.
- The carboxylation of one molecule of RuBP results in two molecules of 3-PGA, thus requiring four NADH in the subsequent coupling reactions. The rate of RuBP consumption (µmol s^{-1}) in the assay volume, is therefore calculated by:

$$RuBPconsumption = (Slope \times Volume)/(6.22 \times 4 \times Pathlength)$$

where the *Slope* represents the change in absorbance per second in the linear part of the absorbance trace change, *Volume* is the final volume per well in mL (0.2), 6.22 is the extinction coefficient of NADH in μmol^{-1} mL cm^{-1} and the factor 4 is used to account for the four molecules of NADH which are oxidized per molecule of RuBP. The *Pathlength* of the assay mix contained in each well is measured in cm.

- Rubisco initial (*Vi*) and total (*Vt*) activity expressed on a leaf area basis (µmol m^{-2} s^{-1}) is then calculated by:

$$V_i or V_t = (RuBPconsumption \times Extraction)/(Leafarea \times Aliquot)$$

where the *Extraction* is the volume of buffer in mL used for leaf extraction, leaf area is in m^2, and *Aliquot* is the volume of leaf extract supernatant used in the assay in mL.

- Rubisco activity can also be expressed on a Rubisco or total soluble protein (TSP) content basis (µmol $min^{-1}mg^{-1}$):

$$V_i or V_t = (RuBPconsumption \times 60)/(Protein \times Aliquot)$$

where 60 is to convert seconds to minutes, *Protein* is the Rubisco or TSP content in mg mL^{-1}, and *Aliquot* is the volume of leaf extract supernatant used in the assay in mL.

- From the Rubisco activity calculations above for initial (*Vi)* and total activity (*Vt*), the Rubisco activation state (AS, %) can be calculated:

$$AS = 100 \times V_i/V_t$$

Note

Measured absorbance values in a microtiter plate need to be normalized to a 1 cm pathlength, which would be found in a typical cuvette used in spectrophotometers. Measurements are corrected using Lambert-Beer's Law and considering both the volume in each well and the specific well dimensions for each type of microtiter plate. Modern microtiter plate readers frequently include a pathlength correction option, but this feature normally does not consider the properties of the solution. It is important to use the respective assay mix in determining the pathlength correction factor as the meniscus will affect the pathlength and absorbance reading in the microtiter plate. The pathlength can be determined according to Lampinen et al., (2012). Please, check the SI information in the publication linked to this protocol for more details.

Citations

Kane HJ, Wilkin JM, Portis AR, Andrews TJ. Potent inhibition of ribulose-bisphosphate carboxylase by an oxidized impurity in ribulose-1,5-bisphosphate
https://doi.org/10.1104/pp.117.3.1059

Kubien DS, Brown CM, Kane HJ. Quantifying the amount and activity of Rubisco in leaves
https://doi.org/10.1007/978-1-60761-925-3_27

Lampinen J, Raitio M, Perälä A, Oranen H, Harinen R. Microplate based pathlength correction method for photometric DNA quantification assay
https://assets.thermofisher.com/TFS-Assets/LCD/Application-Notes/AN-SkanIT-Microplate-Based-Pathlength-Correction-Technical-Note

Sales CRG, Degen GE, Silva AB, Carmo-Silva E. Spectrophotometric determination of Rubisco activity and activation state in leaf extracts
https://doi.org/10.1007/978-1-4939-7786-4_14

Sharwood RE, Sonawane BV, Ghannoum O, Whitney SM. Improved analysis of C4 and C3 photosynthesis via refined in vitro assays of their carbon fixation biochemistry
https://doi.org/10.1093/jxb/erw154

Wong C-H. Practical enzymatic syntheses of ribulose 1,5-bisphosphate and ribose 5-phosphate
https://doi.org/10.1021/ja00547a023