Plant ribulose-1,5-bisphosphate carboxylase/oxygenase(RuBisCO) ELISA Kit

Catalog Number. CSB-E16686Pl

For the quantitative determination of endogenic ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) concentrations in plant tissues.

This package insert must be read in its entirety before using this product.

If You Have Problems

Technical Service Contact information

Phone: 86-27-87582341
Fax: 86-27-87196150
Email: tech@cusabio.com
Web: www.cusabio.com

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

PRINCIPLE OF THE ASSAY

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with antigen. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific for RuBisCO. The competitive inhibition reaction is launched between with pre-coated RuBisCO and RuBisCO in samples with the antibody. Then add a Horseradish Peroxidase (HRP) conjugated goat-anti-rabbit IgG antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of RuBisCO in the sample. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

3.12 μg/ml-800 μg/ml.

SENSITIVITY

The minimum detectable dose of plant RuBisCO is typically less than 3.12 μg/ml. The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest plant RuBisCO concentration that could be differentiated from zero.

SPECIFICITY

This assay has high sensitivity and excellent specificity for detection of plant RuBisCO. No significant cross-reactivity or interference between plant RuBisCO and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between plant RuBisCO and all the analogues, therefore, cross reaction may still exist.

PRECISION

Intra-assay Precision (Precision within an assay): CV%<10%
Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<20%
Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Reagents	Quantity
Assay plate	1(96 wells)
Standard	2 x 500 µl
Antibody (100 x concentrate)	1 x 60 µl
HRP-conjugate(100 x concentrate)	1 x 120 µl
Antibody Diluent	1 x 10 ml
HRP-conjugate Diluent	1 x 20 ml
Sample Diluent	2 x 20 ml
Sample Extraction Buffer (25 x concentrate)	1 x 20 ml
Wash Buffer (25 x concentrate)	1 x 20 ml
TMB Substrate	1 x 10 ml
Stop Solution	1 x 10 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

STORAGE

<table>
<tr><td>Unopened kit</td><td colspan="2">Store at 2 - 8°C. Do not use the kit beyond the expiration date.</td></tr>
<tr><td rowspan="9">Opened kit</td><td>Coated assay plate</td><td>May be stored for up to 1 month at 2 - 8°C. Try to keep it in a sealed aluminum foil bag, and avoid the damp.</td></tr>
<tr><td>HRP-conjugate</td><td rowspan="2">May be stored for up to 1 month at 2 - 8° C. If don’t make recent use, better keep it store at -20°C.</td></tr>
<tr><td>Antibody</td></tr>
<tr><td>Standard</td><td rowspan="8">May be stored for up to 1 month at 2 - 8°C.</td></tr>
<tr><td>Antibody Diluent</td></tr>
<tr><td>HRP-conjugate Diluent</td></tr>
<tr><td>Sample Diluent</td></tr>
<tr><td>Sample Extraction Buffer</td></tr>
<tr><td>Wash Buffer</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

***Provided this is within the expiration date of the kit.**

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100 mL and 500 mL graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.
- Lyophilizer
- Stirrer

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

Weigh 2 grams of fresh clean green leaf tissue. Add 10ml Sample Extraction Buffer and grind with mortar. Transfer to an Eppendorf-vial, stay at 4°C for 30 minutes. Centrifuge for 10 minutes at 5000rpm. Collect the supernatant for testing.

SAMPLE PREPARATION

Samples require a 5-fold dilution into Sample Diluent. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.

Note:

1. CUSABIO is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid loss of bioactivity and contamination.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
5. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.

REAGENT PREPARATION

Note:

1. **Kindly use graduated containers to prepare the reagent.**
2. Bring all reagents to room temperature (18-25°C) before use for 30min.
3. Prepare fresh standard for each assay. Use within 4 hours and discard after use.
4. Making serial dilution in the wells directly is not permitted.
5. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10μl for once pipetting.
6. Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

- **Antibody (1x) -** Centrifuge the vial before opening.
  Antibody requires a 100-fold dilution. A suggested 100-fold dilution is 10μl of **Antibody** + 990 μl of **Antibody Diluent**.

- **HRP-conjugate (1x) -** Centrifuge the vial before opening.
  HRP- conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 μl of **HRP- conjugate** + 990 μl of **HRP- conjugate Diluent**.

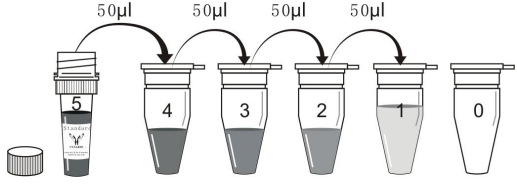
- **Sample Extraction Buffer(1x)-** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Sample Extraction Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Sample Extraction Buffer(1x).

- **Wash Buffer(1x)-** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

- **Standard**

  Centrifuge the standard vial at 6000-10000rpm for 30s. Allow the stock standard solution of 800μg/ml to sit for a minimum of 30 minutes to warm to room temperature prior to making dilutions.

  Pipette 150 μl of **Sample Diluent** into each tube (S0-S4). Use the stock solution(S5) to produce a 4-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (800 μg/ml). **Sample Diluent** serves as the zero standard (0 μg/ml).



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

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a **Blank** well without any solution. Add 50µl of **Standard** or **Sample** per well. Standard need test in duplicate.
4. Add 50µl of **Antibody(1x)** to each well(not to Blank well). Mix well and then incubate for 60 minutes at 37°C.
5. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with **Wash Buffer** (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100µl of **HRP-conjugate(1x)** to each well(not to Blank well). Mix well and then incubate for 60 minutes at 37°C.
7. Repeat the aspiration/wash process for five times as in step 5.
8. Add 90µl of **TMB Substrate** to each well, mix well. Incubate for 20 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
9. Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the

readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding TMB

Substrate (e.g. observation once every 10 minutes), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

6. TMB Substrate is easily contaminated. TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

CALCULATION OF RESULTS

Using the professional soft "Curve Expert" to make a standard curve is recommended, which can be downloaded from our web.

Average the duplicate readings for each standard and sample and subtract the average optical density of Blank.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. 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. The data may be linearized by plotting the log of the RuBisCO concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

植物 1,5-二磷酸核酮糖羧化酶／加氧酶（RuBisCO）酶联免疫试剂盒使用说明书

【产品编号】CSB-E16686Pl

【预期应用】ELISA 法定量测定植物组织裂解液中内源性 RuBisCO 含量。

【产品性能指标】

1、 检测范围：3.12 μg/ml - 800 μg/ml

2、 灵敏度：3.12 μg/ml

3、 精密度：批内差 CV%<10%，批间差 CV%<20%

4、 特异性：本试剂盒特异性检测植物 RuBisCO，且与其他相关蛋白无交叉反应。

【实验原理】

用 RuBisCO 偶联物包被微孔板，制成固相载体，往包被 RuBisCO 偶联物的微孔中依次加入标本或标准品、抗 RuBisCO 抗体。游离 RuBisCO 与微量反应板上的 RuBisCO 偶联物竞争结合抗 RuBisCO 抗体。然后加入 HRP 标记的二抗，经过彻底洗涤后用底物 TMB 显色。TMB 在过氧化物酶的催化下转化成蓝色，并在酸的作用下转化成最终的黄色。颜色的深浅和样品中的 RuBisCO 呈负相关。用酶标仪在 450nm 波长下测定吸光度（OD 值），计算样品浓度。

【所需试剂和器材】

标准规格酶标仪；高速离心机；电热恒温培养箱；干净的试管和 Eppendof 管；容量瓶；系列可调节移液器及吸头；多通道移液器；冷冻干燥机；研磨钵；搅拌器；蒸馏水 等

【试剂盒组成成分】

组份	96T
酶标板 (Assay plate)	96 孔
标准品 (Standard)	2 x 500 µl/瓶
抗体(Antibody)	1 x 60 µl/瓶 (100 x)
酶结合物(HRP-conjugate)	1 x 120 µl/瓶 (100 x)
抗体稀释液(Antibody Diluent)	1 x 10 ml/瓶
酶结合物稀释液(HRP-conjugate Diluent)	1 x 20 ml/瓶
样品稀释液 (Sample Diluent)	2 x 20 ml/瓶
植物组织萃取液(Sample Extraction Buffer)	1 x 20 ml/瓶 (25 x)
浓洗涤液 (Wash Buffer)	1 x 20 ml/瓶 (25 ×)
底物溶液 (TMB Substrate)	1 x 10 ml/瓶
终止液 (Stop Solution)	1 x 10 ml/瓶
板贴	4
说明书	1

【存储条件及有效期】

<table>
<tr><td>未开封试剂盒</td><td colspan="2">试剂盒避光保存于2-8℃。有效期为六个月。
请在试剂盒标注的有效日期内使用。</td></tr>
<tr><td rowspan="11">开封试剂盒</td><td>预包被的酶标板</td><td>酶标板打开后应置有干燥剂的铝箔袋中置于 2-8℃密封防潮保存。有效期内 2-8℃条件下最多可保存一个月。</td></tr>
<tr><td>酶结合物</td><td rowspan="2">有效期内2-8℃条件下最多可保存一个月。若近期不使用，最好保存在-20°C。</td></tr>
<tr><td>抗体</td></tr>
<tr><td>标准品</td><td rowspan="8">有效期内2-8℃条件下最多可保存一个月。</td></tr>
<tr><td>抗体稀释液</td></tr>
<tr><td>酶结合物稀释液</td></tr>
<tr><td>植物组织萃取液</td></tr>
<tr><td>样本稀释液</td></tr>
<tr><td>浓洗涤液</td></tr>
<tr><td>底物溶液</td></tr>
<tr><td>终止液</td></tr>
</table>

【样本采集及保存】

称取 2 克新鲜干净绿叶组织，加入植物组织萃取液，用研钵研磨，样本提取液总体积为 10ml。然后将研磨后的绿叶组织转移至离心管，4℃避光静置 30 分钟。5000rpm 离心 10 分钟。取上清检测。

【样本稀释】

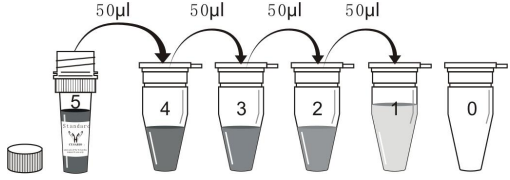
样本用样本稀释液进行 1:5 倍稀释后进行检测。此推荐稀释倍数仅供参考，用户应根据实验自行确定其最优稀释倍数。

【试剂配制】

1、 标准品

(1) 从试剂盒中取出一支标准品，于 6000-10000rpm 离心 30 秒。室温至少放置 30 分钟，备用。此标准品标记为 S5。

(2) 取 5 个 1.5 ml 离心管(S0-S4)依次排列，各加入 150μl 样本稀释液。吸取 50μl 标准品 S5 到第一个离心管中(S4)，轻轻吹打混匀。从 S4 中吸取 50μl 到第二个 EP 管中(S3)，轻轻吹打混匀。以此类推进行标准品的 4 倍稀释。S0 为样本稀释液。



【标准品浓度】

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

2、 洗液工作液

洗液需提前配制，稀释前根据预先计算好的每次实验所需的总量配制。取出洗液浓缩液，浓洗涤液低温保存会有盐析出，稀释时可在水浴中加温助溶。先用量筒量出24ml去离子水，倒入烧杯。取1ml浓洗涤液，均匀加入，摇晃混匀。

3、 抗体工作液

打开瓶盖前请离心，收集瓶壁上的溶液。临用前以抗体稀释液稀释，稀释前根据预先计算好的每次实验所需的总量配制（每孔50μl），实际配制时应多配制0.1-0.2ml。如10μl抗体加990μl抗体稀释液的比例配制，轻轻混匀，在使用前一小时内配制。

4、 酶结合物工作液

打开瓶盖前请离心，收集瓶壁上的溶液。临用前以酶结合物稀释液稀释，稀释前根据预先计算好的每次实验所需的总量配制（每孔100μl），实际配制时应多配制0.1-0.2ml。如10μl酶结合物加990μl酶结合物稀释液的比例配制，轻轻混匀，在使用前一小时内配制。

5、 植物组织萃取液工作液

植物组织萃取液需提前配制，稀释前根据预先计算好的每次实验所需的总量配制。取出植物组织萃取液浓缩液。先用量筒量出24ml去离子水，倒入烧杯。取1ml植物组织萃取液，均匀加入，摇晃混匀。

【注意事项】

1. 实验开始前，请提前配置好所有试剂，试剂或样品稀释时，均需混匀，混匀时尽量避免起泡。
2. 用户在初次使用试剂盒时，应将各种试剂管离心数分钟，以便试剂集中到管底。
3. 在配制标准品、检测溶液工作液时，请以相应的稀释液配制，不能混淆。
4. 标准品、抗体工作液、酶结合物工作液请依据所需的量配置使用。请勿重复使用已稀释过的标准品、抗体工作液、或酶结合物工作液。
5. 标准品应于临用前 15 分钟内配制，用完丢弃，下次检测使用新鲜配置的标准品。
6. 如样品浓度过高时，用样品稀释液进行稀释，以使样品符合试剂盒的检测范围。

【操作步骤】

1. 将各种试剂移至室温（18-25℃）平衡至少半个小时，按前述方法配制试剂，备用。
2. 设置一个空白孔，不加任何溶液。加样：分别设标准孔、待测样品孔。每孔分别加标准品或待测样品 50μl。
3. 每孔加抗体工作液 50μl（空白孔不加），轻轻晃动混匀，覆上板贴，37℃反应 60 分钟。
4. 弃去孔内液体，甩干，洗板 3 次。每次浸泡 2 分钟，200μl/孔，甩干。
5. 每孔加酶结合物工作液 100μl（空白孔不加），覆上新的板贴，37℃温育 60 分钟。

6. 弃去孔内液体，甩干，洗板 5 次。每次浸泡 2 分钟，200μl/孔，甩干。

7. 依序每孔加底物溶液 90μl，37℃避光显色 20 分钟。

8. 依序每孔加终止溶液 50μl，终止反应。

9. 在反应终止后 5 分钟内用酶标仪在 450nm 波长依序测量各孔的光密度（OD 值）。

【操作要点】

1、 为保证检测结果的准确性，建议标准品及样本均设双孔测定。每次检测均需做标准曲线。

2、 **加样**：加样时，请使用一次性的洁净吸头，避免交叉污染。加样时应尽量轻缓，避免起泡，将样本加于酶标板孔底部，切勿沿孔壁加样。一次加样时间最好控制在 10 分钟内，如标本数量多，推荐使用排枪加样。

3、 **温育**：为防止样本蒸发或污染，温育过程中酶标板必须覆上板贴，实验过程中酶标板应避免处于干燥的状态。温育过程中应随时观察温箱温度是否恒定于 37℃，及时调整。温育过程中，温箱不易开启太多次，以免影响温度平衡。

4、 **洗涤**：洗涤过程非常重要，不充分的洗涤易造成假阳性。

(1) 手工洗板方法：吸去（不可触及孔壁和孔底）或甩掉酶标板内的液体；在实验台上铺垫几层吸水纸，酶标板朝下用力拍几次；将推荐的洗涤缓冲液按 200μl/孔注入孔内，浸泡 2 分钟。根据操作步骤中所述，重复此过程数次。

(2) 自动洗板：如果有自动洗板机，应在熟练使用后再用到正式实验过程中。

5、 **显色**：为保证实验结果的准确性，底物反应时间到后应尽快加入终止液。可在加入底物溶液后每隔一段时间观察一下显色情况以控制反应时间（比如每隔 10 分钟）。当肉眼可见标准品前 3-4 孔有明显梯度蓝色，后 3-4 孔显色不明显时，即可加入终止液终止反应，此时蓝色立刻变为黄色。终止液的加入顺序应尽量与底物溶液的加入顺序相同。

6、 底物溶液应为浅蓝色或无色，如果颜色严重变深则必须弃用。底物溶液易受污染，请避光妥善保存。

【数据处理】

可将标准品及样本值减去空白孔数值后绘制曲线，如果设置复孔，则应取其平均值计算。以标准品的浓度为纵坐标（对数坐标），OD 值为横坐标（对数坐标），在对数坐标纸上绘出标准曲线。推荐使用专业制作曲线软件进行分析，可从我们的网站下载专业软件"Curve Expert"，并根据提示制作标准曲线。根据样本 OD 值，由标准曲线查出相应的浓度；或用标准品的浓度与 OD 值计算出标准曲线的回归方程式，将样本的 OD 值代入方程式，计算出样本浓度。若样本检测前进行过稀释，最后计算时需乘以相应的稀释倍数，即为样本的实际浓度。

【说明】

1、 本试剂盒仅供研究使用。

2、 中、英文说明书可能会有不一致之处，请以英文说明书为准。

3、 不同批号试剂不能混用。不要用其它生产厂家的试剂替换试剂盒中的试剂。

4、 刚开启的酶标板孔中可能会含有少许水样物质，此为正常现象，不会对实验结果造成任何影响。

Notes