

QuantiPlate™ Kit for Roundup Ready® Soybean and Soy Flour

Catalog Number AP 032

Highlights:

- Sensitivity: Lowest standard is 0.1% Roundup Ready soy in grain/flour
- Results in one hour

Contents of Kit:

- 12 strips of 8 antibody-coated wells each, in plate frame
- CP4-Enzyme Conjugate
- 1 packet of Buffer Salts
- Substrate
- Stop Solution
- Soy Powder Standards
 - RR Negative soy powder Standard
 - 0.1% RR soy powder Standard
 - 1.0% RR soy powder Standard
 - 2.0% RR soy powder Standard

¹ European Commission Joint Research Centre, Institute for Reference Materials and Measurements, Retieseweg, B-2440 Geel, Belgium. www.irmm.jrc.be

Intended Use

The EnviroLogix QuantiPlate Kit for Roundup Ready Soybean and Soy Flour is a 96-well plate kit designed for the qualitative or quantitative laboratory detection of CP4-EPSPS enzyme (CP4) coded for by the Roundup Ready gene in soybean **grain** or **flour**. This kit has not been validated for—and should not be used with—soy **meal** or any other soy product. This test will detect the CP4 enzyme in 0.1% Roundup Ready (RR) soy grain or flour, and requires one hour to run.

How the Test Works

This Kit is a “sandwich” Enzyme-Linked ImmunoSorbent Assay (ELISA).

In the test, soy grain or flour sample extracts are added to test wells coated with antibodies raised against CP4. Any CP4 present in the sample extract binds to the antibodies, and is then detected by addition of enzyme (horseradish peroxidase)-labeled CP4 antibody.

After a simple wash step, the results of the assay are visualized with a color development step. Color development increases with increasing CP4 sample concentration from 0.1% to 2.0% RR soy, then plateaus and drops off.

Lighter color = Lower concentration

Darker color = Higher concentration

The QuantiPlate Kit for Roundup Ready Soy is calibrated with 0.1%, 1.0% and 2.0% RR soy powder standards, sourced from the IRMM-JRC¹. Samples are either quantitated by graphing the standard concentration vs. absorbance on a quadratic scale, or judged to contain more or less CP4 enzyme than each standard by comparing sample absorbances to the absorbances of the standard extracts. Additional quantities of soy powder standards are available from EnviroLogix (Cat #CON 032), from Sigma-Aldrich (Fluka), or directly from the JRC.

Limit of Detection

The Limit of Detection (LOD) of this Kit is 0.02% Roundup Ready Soy. The LOD was determined by interpolation at 0.066 OD (optical density, absorbance) units from a RR soy powder standard curve. 0.066 OD units was determined to be 3 standard deviations from the mean of a population of negative soy flour and soybean grain samples.

Limit of Quantification

The Limit of Quantification (LOQ) of this Kit was validated at 0.2% RR soybean grain (quantification at concentrations greater than 0.1% and less than 0.2% Roundup Ready soy may be reliable, but has not been validated). The LOQ was determined by fortifying a population of non-transgenic soybean samples (998 beans) with two RR soybeans. The mean recovery was 119.1% with a coefficient of variation [CV, (standard deviation/mean) x 100] of 16.4 %.

Fortification and Recovery

Ground soybean samples prepared at the percentages of Roundup Ready soy indicated below were analyzed by a total of 6 operators (5% RR soy samples required a 1:10 dilution of extract in order to bring the sample onto the standard curve). Recoveries and CVs are indicated for each concentration.

Soy Sample (%RR)	Number of Data Points	Recovery (%)	CV (%)
0	99	all negative	not applicable
0.1	84	93.2	39
0.2	75	119.1	16.4
0.5	12	123.1	19.7
1	12	110.6	18.6
1.5	12	85.9	6.9
2	21	104.2	16.8
5	10	104.6	18.4

Non-Specific Interferences

Testing ground soy samples contaminated with the following non-transgenic crops will not adversely affect results from this plate kit:

Barley, canola, corn, cotton, rice, sorghum, sugarbeet or wheat at up to 100% contamination.

IMPORTANT NOTE: The presence of Roundup Ready corn (Event 603) contaminating a soybean sample MAY cause a positive result in this assay.

Items Not Provided

- disposable tip, adjustable air-displacement pipette to deliver 20 to 1000 microliters (µL)
- distilled or deionized water for preparing Wash Buffer, and for extracting samples
- glass bottle or flask with 1 liter capacity for preparation of Wash Buffer
- grinder or mill capable of reducing samples to a 40-mesh particle size
- test or centrifuge tubes for extraction of grain and dilution of sample extracts
- centrifuge capable of 5000 x g
- multi-channel pipette to deliver 50 µL and 100 µL (optional)
- marking pen (indelible)
- tape or Parafilm®
- timer
- microtiter plate or strip reader with 450 nm filter, and linearity of absorbance to at least 2.5
- wash bottle, or microtiter plate or strip washer
- racked dilution tubes for loading samples into the plate with a multi-channel pipette (optional)



Prepare wash buffer solution

USDA Websites

<http://archive.gipsa.usda.gov/reference-library/handbooks/grain-insp/grbook1/bk1.pdf>

USDA Grain Inspection Handbook, Book 1, Grain Sampling. This document provides a comprehensive overview of recommended sampling guidelines for static lots and grain streams. It reviews the various types of equipment and strategies that can be used to obtain a representative grain sample from different types of containers.

<http://archive.gipsa.usda.gov/biotech/sample1.htm>

Practical Application of Sampling for the Detection of Biotech Grains. This one-page application guide provides a table that gives sample sizes for selected lot concentrations and probability of rejecting the specified concentrations. It also provides a formula for making the calculation for other combinations.

<http://archive.gipsa.usda.gov/biotech/sample2.htm>

Guidance document entitled Sampling for the Detection of Biotech Grains, which provides important statistical sampling considerations when testing for the presence of biotech grains. It covers the basis for making probability determinations in accepting lots based upon different assumptions with respect to sample size, number of samples, sample preparation, etc.

<http://archive.gipsa.usda.gov/biotech/samplingplan1.xls>

This website provides a simple to use Sample Planner (29k Excel Spreadsheet). The planner allows you to enter different assumptions in terms of sample size, number of samples, acceptable quality level and to determine the probability of accepting lots with given concentration levels. It also plots the probabilities in graph form for easy interpretation. Specific data can be saved for documentation and future analyses.

Preparation of Solutions

Wash Buffer

Add the contents of the packet of Buffer Salts (phosphate buffered saline, pH 7.4 - Tween 20) to 1 liter of distilled or deionized water, and stir to dissolve. Store refrigerated when not in use; warm to room temperature prior to assay.

Standard Extracts

The Negative (0), 0.1, 1.0 and 2.0% RR soy powder standards must be extracted prior to performing the test. Standard extracts are then aliquoted and frozen for use in later testing. Procedure:

1. Add 10 mL of water (distilled or deionized) to each 0.2 gram vial of soy powder Standard. Shake or vortex vigorously for 30 seconds, let stand for 1 hour, then shake again.
2. Centrifuge the extracts at 5000 x g for 5 minutes.
3. Pour the clarified extracts into a clean tube, and transfer 0.25 mL aliquots to suitable plastic, labeled, capped tubes for freezer storage (-20°C). These frozen extracts are stable for at least 6 months in a non-defrosting freezer.

Sample Extraction and Preparation

This protocol calls for a small sample (20 to 50 grams) to be analyzed. It is essential that this sample be well mixed and representative of the larger bulk. The test will detect 0.1% RR soy in soy flour, or 1 RR bean in a sample of 999 non-transgenic beans.

NOTE: Thorough mixing of the bulk grain sample and determination of an appropriate sampling plan are critical to the results of this testing, and are the responsibility of the user of this test kit. The USDA/GIPSA has prepared several guidance documents to address the issues involved in obtaining representative grain samples from static lots—such as trucks, barges, and railcars—and for taking samples from grain streams. Sampling plans should be chosen that best meet the needs of both the buyer and seller in terms of acceptable risks. Increasing the number of kernels in the sample and taking multiple samples will increase the likelihood of obtaining representative samples, and maximize the probability of detecting any contamination in the grain lot. For further information on USDA/GIPSA guidelines for obtaining representative samples and assessing detection probabilities for biotech grain, see the websites listed to the left.

It is the responsibility of the user to ensure proper sampling and thorough mixing prior to analysis. Once representative samples have been obtained from the truck or container, they can be reduced in size using a splitter and uniformly ground and mixed.

The finer the grind, the faster and more efficient the extraction. The standards provided are a soy powder. In order for soybean samples to be measured against these standards, the ground/milled samples must be passed through a 40-mesh sieve. The fine sieved material is then extracted and tested. Failure to follow this procedure will result in falsely low reports of sample concentration.

For 1000 bean samples grind in a 32 ounce “Mason” jar for 1 minute, on a blender at high speed. Shake jar to mix, then repeat the grinding a second time. Alternatively, pass through an appropriate mill.



Grind soybeans



Clarify extract



Allow all reagents to reach room temperature before beginning



Remove unneeded strips

NOTE: Thoroughly clean the grinding equipment between each sample to avoid cross-contamination.

1. Pour the entire ground sample onto a 40-mesh sieve. Sieve until a 20 to 50 gram sample has passed through. Weigh at least 20 grams of sieved ground soy sample into a jar or cup.
2. Add 100 mL of water to each 20 gram sample. For all other sample sizes, add water at the rate of 5 mL per gram of grain. Cap and shake vigorously by hand or vortex for 20-30 seconds. Let stand at room temperature for one hour to extract. Mix again at the end of the hour.
3. Clarify the extracts by centrifuging at 5000 x g for 5 minutes. Insert a pipette tip below any floating lipid layer and above the precipitate to remove the clarified sample.

Dilution of Standard and Sample Extracts:

Standard Extracts

1. Thaw a vial of each of the frozen standard extracts (prepared according to the instructions on page 3): 0, 0.1, 1.0 and 2.0% RR.
2. Dilute each standard 1:5 in Wash Buffer: mix 100 μ L extract plus 400 μ L Wash Buffer.

NOTE: Thawed standard extracts should be used within 48 hours, and refrigerated when not in use.

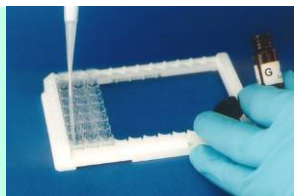
Sample Extracts

1. Dilute each sample extract 1:50 in Wash Buffer: mix 20 μ L clarified extract in 980 μ L Wash Buffer. Sample extracts must be analyzed on the day they were extracted.

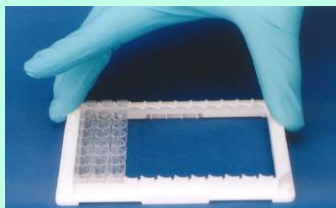
Standards and samples are now ready to be added to the assay plate.

How to Run the Assay

- Read all of these instructions before running the kit.
 - Allow all reagents to reach room temperature before beginning (at least 30 minutes with un-boxed strips and reagents at room temperature - do not remove strips from bag with desiccant until they have warmed up).
 - If more than three strips are to be run at one time, the loading time will most likely exceed 10 minutes, and the use of a multi-channel pipette is strongly recommended in steps 3, 6 and 8.
 - If three or fewer strips are to be run, use a disposable-tip, air-displacement pipette and a clean pipette tip to add each Standard and sample extract to the wells. Conjugate, Substrate, and Stop Solution may be added in the same manner; alternatively, use a repeating pipette with a disposable tip for these three reagents.
 - If fewer than all twelve strips are used, reseal the remaining strips and the desiccant in the foil pouch, and refrigerate.
 - Use the well identification markings on the plate frame as a guide when adding the samples and reagents. For a quantitative assay, the four Standards (C0-C3), along with 44 sample extracts (S), may be run in duplicate wells on one plate. Refer to Figure 1 for a quantitative assay example plate layout.
1. Once all components have reached room temperature, remove the plate from the pouch. Return unneeded strips to pouch and reseal.



Add Standard and sample extracts



Mix plate



Bottle Wash method



Strip Washer method



Complete protocol and add Stop Solution

2. Organize Enzyme Conjugate, all Standard extracts, sample extracts, and pipettes so that Step 3 can be performed in 10 minutes or less.
3. Add **50 µL of Roundup Ready Enzyme Conjugate** to each well, followed immediately by **50 µL** of diluted **0, 0.1%, 1.0% and 2.0% RR Standard extracts (C0-C3)**, and **50 µL** of each diluted **sample extract (S)** to their respective wells, as shown in the Example Plate Layout (Figure 1).
4. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the bench top for 20-30 seconds. Use care when mixing to avoid cross-contamination. Cover the wells with tape or Parafilm and **incubate at ambient temperature for 45 minutes**.
5. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a sink or other suitable container. Flood the wells completely with Wash Buffer, then shake to empty. Repeat this wash step three times. Alternatively, perform these four washes (300 µL/well) with a microtiter plate or strip washer. Slap the inverted plate on a paper towel to remove as much liquid as possible.
6. Add **100 µL of Substrate** to each well.
7. Thoroughly mix the contents of the wells, as in step 4. Cover the wells with new tape or Parafilm and **incubate for 15 minutes** at ambient temperature.

Caution: Stop Solution is 1.0 N Hydrochloric acid. Handle carefully.

8. Add **100 µL of Stop Solution** to each well and mix thoroughly. This will turn the well contents yellow.

NOTE: Read the plate within 30 minutes of the addition of Stop Solution.

How to Interpret the Results

Spectrophotometric Measurement

1. Set the wavelength of the microtiter plate reader to 450 nanometers (nm). If it has dual wavelength capability, use 600, 630 or 650 nm as the reference wavelength.
2. Set the plate reader to blank on the RR Negative soy powder Standard wells (this should automatically subtract the mean optical density (OD) of the RR Negative soy powder Standard wells from each other Standard and sample OD). If the reader cannot do this, it must be done manually.
3. General test criteria:

The mean OD of the RR Negative soy powder Standard wells should not exceed 0.2. The coefficient of variance (%CV) of the duplicate Standard and sample wells should not exceed 15%:

$$\%CV = \frac{\text{std. deviation of ODs} \times 100}{\text{Mean OD}}$$

4. For a quantitative assay, a quadratic (or polynomial) curve fit for the standard curve should be used if the microtiter plate reader you are using has data reduction capabilities. If not, calculate the results manually as described in the "How to Calculate the Quantitative Results" section.

NOTE: Soy samples containing more than 10% Roundup Ready soy may show decreasing ODs with increasing concentration. Do not attempt to extrapolate sample concentrations beyond the range of the standard curve generated in this kit.



Read plates in a Plate Reader within 30 minutes of the addition of Stop Solution

How to Interpret the Qualitative Results

Compare the ODs of the sample extracts to those of the Standards to obtain an estimate of the % RR sample. Samples with ODs greater than that of the lowest standard are considered positive. Those with OD's lower than that of the lowest standard either contain no, or less than 0.1%, Roundup Ready Soy.

How to Calculate the Quantitative Results

1. After reading the wells, average the OD of each set of Standards and samples, and subtract the average OD of the RR Negative soy powder Standard wells from all (if your reader has not automatically done so).
2. Graph the mean OD of each Standard against its % RR content with a quadratic curve fit (see Figure 3).
3. Determine the % RR content of each sample by finding its OD value and the corresponding concentration level on the graph.
4. Interpolation of sample concentration is only possible if the OD of the sample falls within the range of OD's of the Standards.

If the OD of a sample is lower than that of the lowest Standard (0.1% RR), the sample must be reported as less than 0.1% RR Soy.

If the OD of a sample is higher than that of the highest Standard (2.0% RR), the sample must be reported as greater than 2% RR Soy.

If a concentration must be determined for these high level samples, dilute the sample extract 1:10 more than executed in the original assay, in Wash Buffer. Run this dilution in a repeat of the assay. If the result now falls within the range of the OD's of the Standards, multiply the results from the standard curve by 10.

Figure 3. Illustrative standard curve

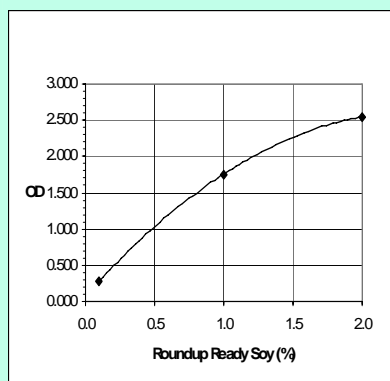


Figure 1. Example of a typical Quantitative assay setup.

	1	2	3	4	5	6	7	8	9	10	11	12
A	C0	C0	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
B	C1	C1	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
C	C2	C2	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
D	C3	C3	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
E	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
F	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
G	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
H	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44

Figure 2. Illustrative calculations

Well contents	OD	Average OD ± sd	% CV	Roundup Ready Conc. (%)
Negative RR Standard	0.009 / 0.006	0.007 ± 0.002	NA	NA
0.1% RR Standard	0.297* / 0.279	0.288 ± 0.013	4.4	NA
1.0% RR Standard	1.776* / 1.740	1.758 ± 0.025	1.4	NA
2.0% RR Standard	2.610* / 2.493	2.551 ± 0.083	3.2	NA
Sample	0.554* / 0.537	0.545 ± 0.006	2.2	0.25% RR Soy

* Figures are after subtraction of Negative RR Standard values.

Actual values may vary; this data is for demonstration purposes only.



Precautions and Notes

- Store all QuantiPlate Kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not expose QuantiPlate Kit components to temperatures greater than 37°C (99°F) or less than 2°C (36°F).
- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before use.
- Do not use kit components after the expiration date.
- Do not use reagents or test well strips from one QuantiPlate Kit with reagents or test well strips from a different QuantiPlate Kit.
- Do not expose Substrate to sunlight during pipetting or while incubating in the test wells.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.
- The assay has been optimized to be used with the protocol provided in the kit. Deviation from this protocol may invalidate the results of the test.
- Observe any applicable regulations when disposing of samples and kit reagents.



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