QualiPlate™ Kit for Roundup Ready® Corn Event 603 and Cotton

Catalog Number AP 010

Highlights:

- *Will detect 0.1% (1 seed in 1000) of Event 603 corn*
- *Test Roundup Ready corn or cotton seed lot purity in 1 hour*

Contents of Kit:

- *1 antibody-coated 96-well plate*
- *Positive Control ground corn*
- *Roundup Ready Enzyme Conjugate*
- *1 packet of Buffer Salts*
- *Substrate*
- *Stop Solution*

	OD (%CV)	**Pos. Ctl. Ratio** (%CV)
Inter-Assay		**n=33**
0.15%	23.9%	21.1%
0.4%	22.1%	20.8%

Intended Use

The EnviroLogix QualiPlate Kit for Roundup Ready Corn Event 603 and Cotton is designed for the qualitative laboratory detection of CP4 EPSPS enzyme (CP4) coded for by the Roundup Ready gene in Corn Event 603 grain, leaf, or seed, and cotton leaf and single seed. For the detection of Roundup Ready in soy bulk grain or soy flour, please refer to the Product Application Guide (Page 9). This test will detect the CP4 enzyme found in 0.1% Event 603 corn (one seed in 1000) and requires 1 hour to run.

NOTE: In corn, this kit can ONLY be used to detect RR Corn Event 603. It will NOT detect RR corn with the Event known as GA21.

How the Test Works

The EnviroLogix QualiPlate Kit for Roundup Ready Corn Event 603 and Cotton is a "sandwich" Enzyme-Linked ImmunoSorbent Assay (ELISA).

In the test, corn or cotton 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.

Lighter color = Low concentration
Darker color = High concentration

How the Kit Performs

The EnviroLogix QualiPlate Kit for Roundup Ready Corn Event 603 and Cotton is a strictly qualitative (yes/no) assay. Samples are interpreted in comparison with Positive and Negative Control ground corn samples provided in each kit. Instructions for interpreting results based upon these controls start on page 6.

Precision

CP4-fortified control solutions were repetitively analyzed in different assays on different days (inter-assay). The fortification levels used are roughly equivalent to 0.15% and 0.4% Event 603 corn, respectively. The data is expressed as % CV for both the optical density absorbance (OD) and the Positive Control Ratio (OD of sample divided by the OD of the Positive Control ground corn).

Error Rate

Event 603 Corn

Validation of this QualiPlate Kit for corn involved in-house and beta-site (non-EnviroLogix users) components. Five different in-house operators and five different beta-sites participated. Each corn sample extract was tested in three different Plate Kit manufacturing lots, generating 3 data points per corn sample.

1000-kernel seed/grain samples

2 false positive results out of 378 non-Corn Event 603 data points, for a best estimate false positive rate of 0.53%.

2 false negative results out of 360 0.1% Corn Event 603 data points, for a best estimate false negative rate of 0.55%.

Single seed samples

0 false positive results out of 387 non-Corn Event 603 seed data points, for a best estimate 0% false positive rate.

0 false negative results out of 366 Corn Event 603 seed data points, for a best estimate 0% false negative rate.

Single leaf punch samples

0 false positive results out of 378 non-Corn Event 603 leaf data points, for a best estimate 0% false positive rate.

0 false negative results out of 378 Corn Event 603 leaf data points, for a best estimate 0% false negative rate.

IMPORTANT NOTE: The presence of Roundup Ready Soybean in a corn sample WILL cause a positive result in this assay.

Roundup Ready Cotton

Validation of this QualiPlate Kit for cotton involved in-house and beta-site (non-EnviroLogix users) components. Four different in-house operators and five different beta-sites participated. Each cotton sample extract was tested in three different Plate Kit manufacturing lots, generating 3 data points per cotton sample.

Single seed samples

0 false positive results out of 591 non-Roundup Ready cotton seed data points, for a best estimate 0% false positive rate.

7 false negative results out of 555 Roundup Ready cotton seed data points, for a best estimate 1.3 % false negative rate.

Single leaf punch samples

13 false positive results out of 1593 non-Roundup Ready cotton leaf data points, for a best estimate 0.8 % false positive rate.

0 false negative results out of 567 Roundup Ready cotton leaf data points, for a best estimate 0% false negative rate.

Items Not Provided

- distilled or deionized water for preparing Wash/Extraction Buffers
- glass bottles or flask plus graduated cylinder with 1 liter capacity for preparation and storage of Wash/Extraction Buffer
- Tween® 20 (Sigma cat# P 1379, or equivalent), Sodium tetraborate (Borax, Sigma cat# S 9640, or equivalent, optional) for cotton sample extraction
- Waring laboratory blender (model 31BL91 or equivalent), glass jar adapter (Eberbach # E8495) and 32 oz. glass Mason jars for ground corn samples
- Snap-cap tubes and pestles for extraction of leaf samples (EnviroLogix Cat# ACC 002, 100/package)
- centrifuge capable of 5000 x g (optional)
- disposable tip, adjustable air-displacement pipettes which will measure 50 and 100 microliters (µL)

- marking pen (indelible)
- tape or Parafilm®
- timer
- microtiter ELISA plate reader
- wash bottle, or microtiter plate or strip washer
- multi-channel pipette that will measure 50 and 100 µL
- racked dilution tubes for loading samples into the plate with a multi-channel pipette, or the equivalent
- orbital plate shaker (optional)

Preparation of Solutions

Prepare wash buffer and grain extraction solutions

Wash/Extraction Buffer:
Add the contents of the packet of **Buffer Salts** to 1 liter of distilled or deionized water and stir to dissolve. Store refrigerated when not in use; allow to come to room temperature prior to assay. If more Wash/Extraction buffer is needed, order item # P-3563 from Sigma Chemical Co. (St. Louis, MO), or prepare the equivalent. Use this buffer for the wash step of the assay, and to extract all corn samples.

Cotton Extraction Buffers:
Cotton leaf and seed samples may be extracted with either of the following buffers:

PBS-0.55% Tween: Add 0.5 mL Tween 20 to 100 mL Wash/Extraction Buffer. . Store refrigerated when not in use; allow to come to room temperature prior to assay.

Borate-Tween: Prepare 0.1 M sodium tetraborate/0.5% Tween 20 (38.1 grams per liter of de-ionized water plus 5 mL Tween 20). Adjust pH to 7.5. Store refrigerated when not in use; allow to come to room temperature prior to assay.

Sample Preparation

Note: It is recommended that the user prepare known negative and positive seed or leaf samples to be run in every assay as controls, in addition to the kit Positive Control ground corn.

Positive Control ground corn extract:
An extract of this control must be run in every assay. To extract, add 5 mL of Wash/Extraction Buffer to each tube containing 2 grams of ground Control corn. Cap and shake vigorously by hand or vortex for 20-30 seconds. Let stand at room temperature for 1 hour to extract. Mix again at the end of the hour, then clarify by allowing to settle 10 minutes or by centrifuging 5 minutes at 5000 x g.

If running the assay at a later date, or more than one assay per plate, freeze 0.5 mL aliquots of each clarified extract. Thaw just prior to use.

Sampling Ground Corn Grain/Seed
This protocol requires that a small sample (20 to 50 grams) be analyzed. It is essential that this sample be well mixed and representative of the larger bulk. The test will detect 0.1% Event 603 corn (one positive kernel in a sample of 1000 kernels).

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.

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/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/sample1.htm
Practical Application of Sampling for the Detection of Biotech Grains. This one-page applica-tion guide provides a table that gives sample sizes for selected lot concentrations and probability of rejecting the specified concentra-tions. It also provides a formula for making the calculation for other combinations.

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.

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.

Grind and Extract the Samples

Ground Corn Grain/Seed:

Once representative samples have been obtained from a 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.**

1. For 1000 kernel samples, grind in a 32 ounce "Mason" jar on a blender at high speed for 1 minute. Shake jar to mix, then repeat the grinding a second time. Thoroughly clean the grinding equipment between samples to prevent cross-contamination.
2. Weigh at least 20 grams of ground corn sample into a jar or cup.
3. Add 50 mL of Wash/Extraction Buffer to each 20 gram sample. For all other grain sample sizes, add Wash/Extraction Buffer at the rate of 2.5 mL per gram of grain. Cap and shake vigorously by hand or vortex for 20-30 seconds. Let stand at room temperature for 1 hour to extract. Mix again at the end of the hour.
4. For best results, clarify the extracts by centrifuging at 5000 x g for 5 minutes. Alternatively, allow them to settle out for at least 10 minutes. Insert a pipette tip below any floating lipid layer and above the pellet to remove the clarified sample. Dispensing particles into the test plate can cause false positive results.

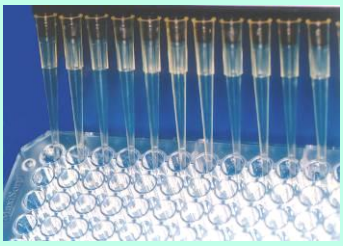
Single Corn or Cotton Seed Samples:

1. Crush seeds: Seeds may be placed in a resealable plastic bag and smashed with a hammer, then transferred to a tube; or, a seed crusher/48-well plate combination may be used (for example Hypure #HSC-100, PerkinElmer, Norton, OH, with Costar plate #3548, Corning Life Sciences, Acton, MA, or equivalent). Check to be sure that all seeds have been crushed. Take extreme care not to cross-contaminate between seed samples. If using the seed crusher, dip the crushing prongs in clean water, then shake off the excess prior to crushing. After crushing, slide a piece of paper between the plate and the crushing prongs as you remove them from the wells. These procedures help to prevent seed particles from jumping from one well to the next, reducing the risk of cross-contamination.
2. Add 1 mL of Wash/Extraction Buffer to each crushed corn seed; add 1 mL of PBS-0.55% Tween or Borate-Tween to each crushed cotton seed. Mix for at least 30 seconds, then allow particles to settle. Dispensing particles into the test plate can cause false positive results.

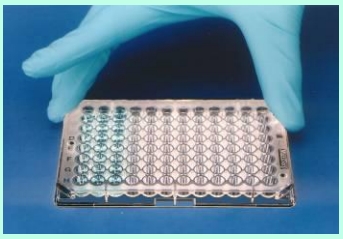
Single Corn or Cotton Leaf Punch Samples:

1. Take a single leaf punch of approximately 5 millimeters diameter, using a micro-tube cap or a paper punch. Mash the leaf tissue with a pestle matched to the micro-tube, or with a disposable pipette tip, or a Hypure cutter (HCT-200, PerkinElmer, Norton, OH) in a 96-well plate (Costar #3370, Corning Life Sciences, Acton, MA, or equivalent).
2. Add 0.25 mL of Wash/Extraction Buffer per corn leaf punch; add 0.25 mL of PBS-0.55% Tween or Borate-Tween to each cotton leaf punch. Mix for at least 30 seconds, then allow particles to settle. Take extreme care not to cross-contaminate between leaf samples. Dispensing particles into the test plate can cause false positive results.

Allow all reagents to reach room temperature before beginning



Add Conjugate, controls and samples



Mix plate

Incubate

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 plates and reagents at room temperature - do not remove plate from bag with desiccant until it has warmed up).
- Organize all Control and sample extracts, and pipettes so that Step 1 can be performed in 15 minutes or less.
- Use the well identification markings on the plate frame to guide you when adding the samples and reagents. For this qualitative assay, duplicate wells of the Wash/Extraction Buffer Blank (BL), user-supplied Negative Control (NC), and the Positive Control (PC), along with 90 sample extracts (S) in single wells may be run on one plate. (See the Qualitative Assay Example Plate Layout - Figure 1a).

Procedure

1. Add **50 µL** of **Roundup Ready Enzyme Conjugat**e to each well, followed immediately by **50 µL of Wash/Extraction Buffer Blank (BL)**, **50 µL** of **Negative and Positive Control ground corn** extracts **(PC and NC)** and **50 µL** of each **sample extract (S)** to their respective wells, as shown in the Example Plate Layout (Figure 1a).

NOTE: In order to minimize setup time it is strongly recommended that a multi-channel pipette be used in steps 1, 5, and 7.

2. Thoroughly mix the contents of the wells by moving the plate in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill the contents!

3. Cover the wells with tape or Parafilm® to prevent evaporation and incubate at ambient temperature for **45 minutes**. If an orbital plate shaker is available, shake plate at 200 rpm.

4. 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/Extraction 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.

5. Add **100 µL** of **Substrate** to each well.

6. Thoroughly mix the contents of the wells, as in step 2. Cover the wells with new tape or Parafilm and **incubate** for **15 minutes at ambient temperature**. Use orbital shaker if available.

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

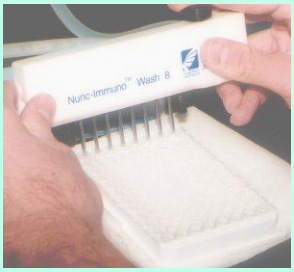
7. Add **100 µL** of **Stop Solution** to each well and mix thoroughly. This will turn the well contents yellow.

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.)

Bottle Wash method



Strip Plate Wash option

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

2. Set the plate reader to blank on the Wash/Extraction Buffer Blank wells (this should automatically subtract the mean optical density (OD) of the Blank wells from each control and sample OD). If the reader cannot do this, it must be done manually.

   General test criteria:

   The mean OD of the BLANK wells should not exceed 0.2.

   The mean, blank-subtracted OD of the Positive Control wells should be at least 0.2 and at least 3x greater than the mean, blank-subtracted OD of the Negative Control wells.

   The coefficient of variance (%CV) between the duplicate Positive Control wells should not exceed 15%:

   $$\%CV = \frac{\text{std. deviation of OD's}}{\text{mean Pos.Ctl. OD}} \times 100$$

If the results of an assay fail to meet these criteria, consult EnviroLogix' Technical Service for suggestions on improving the test when you repeat the assay.

Calculate the Positive Control Ratio

Divide the OD of each sample extract by the mean OD of the Positive Control ground corn extract wells. This number is the "Positive Control Ratio".

Interpret the Qualitative Results

Ground corn samples
If the Positive Control Ratio calculated for a sample is less than 0.25, the ground corn contains less than 0.1% Event 603 corn.

If the Positive Control Ratio of a sample is greater than or equal to 0.25, the sample contains 0.1% or greater Event 603 corn.
NOTE: Ground corn samples containing more than 25% Event 603 corn may show decreasing OD's with increasing concentration. However, the OD's will be much greater than that of a 0.1% Event 603 sample. This test is to be used qualitatively only, with yes/no results at 0.1% Event 603 corn. For information on testing at different cutoff levels, please contact EnviroLogix' Technical Service.

Single Corn or Cotton Leaf and Seed samples:
If the Positive Control Ratio calculated for a sample is less than 1.0, the sample is not Event 603 corn or Roundup Ready cotton.

If the Positive Control Ratio of a sample is greater than or equal to 1.0, the sample is Event 603 corn or Roundup Ready cotton.

Leaf and seed samples are by their nature either 100% positive or 100% negative. Any low level positive results from single seed or leaf samples must be due to either some form of sample cross-contamination (stray particles or dust from Event 603 corn or cotton, leaf residue on leaf punch, etc.) or can be caused by transfer of particulate matter from leaf or seed extracts into the assay wells. If there is any question of the latter occurring, re-extraction and re-testing is recommended.

Figure 1a. Example of a typical Qualitative assay setup.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
B	NC	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
C	PC	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
D	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
E	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
F	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	BL
G	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	NC
H	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	PC

Precautions and Notes

- Store all QualiPlate Kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not expose QualiPlate 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 plates from one QualiPlate Kit with reagents or plates from a different QualiPlate Kit.
- Do not expose Substrate to sunlight during pipetting or while incubating in the test wells.
- 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.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.
- Observe any applicable regulations when disposing of samples and kit reagents.
- Use caution to prevent sample-to-sample cross-contamination with samples, fluids, or disposables.

For Technical Support Contact Us At:

EnviroLogix
500 Riverside Industrial Parkway
Portland, ME 04103-1486
USA

Tel: (207) 797-0300
Toll Free: 866-408-4597
Fax: (207) 797-7533

e-mail:
info@envirologix.com

website:
www.envirologix.com

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LICENSE

EnviroLogix has developed this kit using proprietary reagents as well as reagents licensed from the Monsanto Company.

PRODUCT APPLICATION GUIDE
Testing Roundup Ready Soy Bulk Grain or Flour

Intended Use

This Application Guide provides instruction for the use of the AP 010 QualiPlate Kit for Roundup Ready Corn and Cotton for 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 (RR1) or 0.2% Roundup Ready 2 Yield (RR2) soy grain or flour, and requires one hour to run. Follow instructions in the product insert for running the assay. This guide covers sample preparation, calibration, and data interpretation for the soybean/soy flour matrices.

Materials Required:

- EnviroLogix QualiPlate Kit for Roundup Ready Corn and Cotton (AP 010, or AP 010 NWV10)
- RR1 soy powder standard(s), sourced from the IRMM-JRC (European Commission Joint Research Centre, Institute for Reference Materials and Measurements, Retieseweg, B-2440 Geel, Belgium. www.irmm.jrc.be)
- RR2 soy powder standard (available as 100%), sourced from AOCS (American Association of Oil Chemists, Headquarters 2710 S. Boulder, Urbana, IL 61802-6996 USA; https://secure.aocs.org/crm/index.cfm)
- centrifuge capable of 5000 x g
- 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

Standard Extracts:

Recommended concentrations of RR Standards are Negative (0), 0.1, 1.0 and 2.0% RR1 soy powder, or Negative, 0.2, 2.0, and 4% RR2Y soy powder. These standards must be powders that will pass through a 40-mesh sieve. Mix positive and negative powders by weight to prepare the desired concentrations.

Standards must be extracted prior to performing the test. Standard extracts may then be aliquoted and frozen for use in later testing. Procedure:

1. Add 50 mL of water (distilled or deionized) to each 1 gram 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% RR1 soy in soy flour (or 1 RR bean in a sample of 999 non-transgenic beans) or 0.2% RR2Y soy in soy flour (or 1 RR2Y bean in a sample of 499 non-transgenic beans).

- 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 commercial standards 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.
- Thaw any frozen standard extracts (prepared according to the instructions on page 1).

  NOTE: Thoroughly clean the grinding and sieving 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.
4. Dilute the **sample** extract 1:50 in Wash Buffer: mix 20 µL clarified extract in 980 µL Wash Buffer. Sample extracts must be analyzed on the day they were extracted.
5. Dilute each thawed **standard** extract 1:5 in Wash Buffer: mix 100 µL extract plus 400 µL Wash Buffer. **NOTE:** Thawed standard extracts should be used within 48 hours, and refrigerated when not in use.

Standards and samples are now ready to be added to the assay plate. Follow the instructions as described in the section on page 5 entitled "How to Run the Assay." For a quantitative assay, use duplicate wells for each standard and sample.

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.

1. 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.
2. 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%:

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

3. 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.

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 contain less than 0.1% RR1 Soy or less than 0.2% RR2 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.
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, the sample must be reported as less than 0.1% RR1 soy or 0.2% RR2 soy.

   If the OD of a sample is higher than that of the highest Standard, the sample must be reported as greater than 2% RR1 Soy or 4% RR2 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.