ENVIROLOGIX™

QualiPlate™ Kit for 2mEPSPS

Catalog Number AP 084 NW V10

Highlights:

- *Test 2mEPSPS cotton seed lot purity in 1.5 hour*

Contents of Kit:

- *10 antibody-coated plates*
- *2mEPSPS Antibody-Enzyme Conjugate*
- *Substrate*

Materials Not Provided

- *Wash Buffer (preparation instructions, right)*
- *Extraction Buffer (instructions, right)*
- *Stop Solution (instructions, right)*
- *distilled or deionized water for preparing Wash and Extraction Buffers*
- *user-supplied controls: seed and/or leaf extracts from known negative and positive samples*
- *EnviroLogix Tissue Extraction Kit (ACC 002) or other suitable equipment for taking and extracting leaf punch samples*
- *equipment for pulverizing seeds*
- *disposable tip, adjustable air-displacement multi-channel pipettes which will measure 100 microliters (µL)*
- *marking pen (indelible)*
- *tape or Parafilm®*
- *timer*
- *microtiter plate reader with 450 nm filter*
- *wash bottle, or microtiter plate or strip washer*

Intended Use

The EnviroLogix QualiPlate Kit for 2mEPSPS is designed for the qualitative laboratory detection of 2mEPSPS protein in Glytol® cotton leaf or seed.

NOTE: The 2mEPSPS protein in Glytol® cotton is very similar in structure to the endogenous EPSPS protein expressed in all maize tissue. Corn leaf and seed samples, whether from biotechnology-enhanced seed stocks or conventional, will give a positive result in this test kit.

How the Test Works

This Kit is a "sandwich" Enzyme-Linked ImmunoSorbent Assay (ELISA). In the test, cotton sample extracts are added to test wells coated with antibodies raised against 2mEPSPS protein. Any 2mEPSPS present in the sample extract binds to the antibodies and is then detected by addition of enzyme (horseradish peroxidase)-labeled 2mEPSPS antibody.

After a simple wash step, the results of the assay are visualized with a color development step. Color development increases with increasing 2mEPSPS sample concentration.

Lighter color = Low concentration
Darker color = High concentration

How the Kit Performs

This Kit is a strictly qualitative (yes/no) assay. It is recommended that the user prepare known negative and positive seed or leaf samples to be run in every assay as controls. Samples are interpreted in comparison to Extraction Buffer blanks, and user-supplied negative and positive matrix controls.

Preparation of Solutions

- **Wash/Buffer**: 0.01M Phosphate/0.15M Saline/ 0.05% Tween-20 Solution, pH 7.4. This can be purchased in 1L dry packets from Sigma Chemicals, Cat#P-3563, or prepared from salts and Tween-20 on site. Store at controlled ambient temperature for up to one week, then discard. Use this buffer for the wash step of the assay, and to **extract seed samples** for the qualitative assay. To **extract leaf samples**, add 0.5 mL Tween 20 to 100 mL Wash/Extraction Buffer. Stir to mix, and store as above.
- 1 N Hydrochloric acid (HCl) **Stop Solution**: Prepare by adding 83 mL of concentrated HCl (36.5-38.0%) to 917 mL of distilled or deionized water. Work in a fume hood and use proper protective gear. This reagent may be stored at room temperature for 2 years.

Sample Preparation

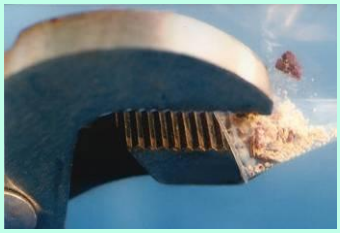
Single Cotton Seed Samples:

1. Crush seeds: Seeds may be crushed by any number of methods, from hammers or pliers in a bag or tube, to 48-well seed crushers, to bead-beater type grinders. Well-extracted seeds should result in a tan cloudy extract. Note the presence of any extracts that appear clear and/or colorless – these may not have extracted properly and assay data would be invalid. For best results, extract another representative sample.

Prepare wash buffer and extraction solutions

Allow all reagents to reach room temperature before beginning

Punch leaf sample



Crush single seed

CAUTION: 2mEPSPS protein is expressed at high concentrations in cotton seed, so there is serious potential for cross-contamination between samples during seed crushing. Use the utmost care to avoid this. Cleaning the cutting/crushing surfaces with an alcohol-soaked pad between samples is recommended.

2. Add 1 mL of **Wash/Extraction Buffer** to each crushed seed. Mix for at least 30 seconds. For best results, allow to extract for at least 10 minutes, mixing again at the end of that time. If seeds are thoroughly crushed, this extraction time can be reduced. Allow extracts to settle completely, or centrifuge. Dispensing particles into the test plate can cause false positive results.

Single Cotton Leaf Punch Samples:

1. Take a single leaf punch of approximately 5 to 10 millimeters diameter, using a paper punch or micro-tube cap. Mash the leaf tissue with a pestle matched to the micro-tube, or disrupt via another method. The extraction efficiency of whatever method used will vary proportionately with the amount of tissue disruption performed.
2. Add 0.25 to 0.5 mL of **Wash/Extraction Buffer plus 0.5% Tween 20** per leaf punch. Mash again with pestle or mix vigorously for at least 30 seconds, and allow solids to settle. Dispensing particles into the test plate can cause false positive results. Take extreme care not to cross-contaminate between leaf samples. Well-extracted leaves should result in a green cloudy extract. Note the presence of any extracts that appear clear and/or colorless – these may not have extracted properly and assay data would be invalid. For best results, extract another representative sample.

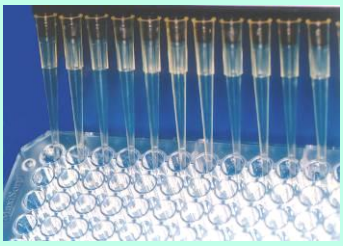
How to Run the Qualitative Assay

- 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 strips 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. The use of a multi-channel pipette is strongly recommended for all reagent additions.
- Use the well identification markings on the plate frame as a guide when adding the samples and reagents. For this qualitative assay, duplicate wells of the Extraction Buffer Blank (BL) and a user-supplied Positive Control (PC), along with 92 sample extracts and user-supplied controls (S) in single wells may be run on one plate. (See the Qualitative Assay Example Plate Layout - Figure 1a).

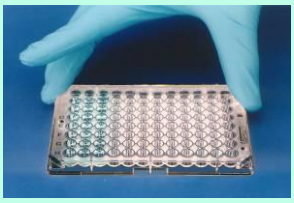
Procedure

1. Add **100 uL** of **2mEPSPS Enzyme Conjugate** to the wells, followed immediately by **100 µL** of **Extraction Buffer Blank (BL)**, **100 µL** of **Positive Control (PC)**, and **100 µL** of each **sample and other user-prepared control extracts (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, 8, and 10.
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**.

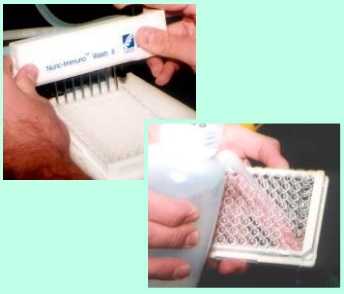


Add controls and samples



Mix plate

Incubate



Strip or Bottle Wash method

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 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**.

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.

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 **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 0.15.

The coefficient of variance (%CV) between any 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 repeating the assay.

Interpret the Qualitative Results

Single Cotton Leaf and Seed samples:

Leaf and seed samples are by their nature either 100% positive or 100% negative. Compare sample absorbances to those of your negative and positive control wells to determine whether a seed or leaf contains the 2mEPSPS protein. Any low level positive results from single seed or leaf samples might be due to either cross-contamination (stray particles or dust from cotton, leaf residue on leaf punch, etc.) or unintended 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.

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

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
B	PC	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
C	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
D	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
E	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
F	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
G	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	BL
H	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	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 Plate Kit with reagents or plates from a different Plate 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 and extraction buffers described in this product insert. 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.

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