QualiPlate™ Kit for Vip3A

Highlights:

Screens single seed or leaf samples for the presence of Vip3A protein in a 1 hour assay

Contents of Kit:

- *10 antibody-coated 96-well plates*
- *Vip3A Enzyme Conjugate*
- *Substrate*



Prepare wash buffer and extraction solutions

Catalog Number AP 085 NWC V10

Intended Use

The QualiPlate Kit for Vip3A is designed for the qualitative laboratory detection of Vip3A protein in individual corn seed or leaf punch samples.

How the Test Works

The QualiPlate Kit is a "sandwich" Enzyme-Linked ImmunoSorbent Assay (ELISA).

In the test, sample extracts are added to test wells coated with antibodies raised against Vip3A protein. Any Vip3A present in the sample extract binds to the antibodies, and is then detected by addition of enzyme (horseradish peroxidase)-labeled Vip3A antibody.

After a simple wash step, the results of the assay are visualized with a color development step; color development is proportional to Vip3A concentration in the sample extract.

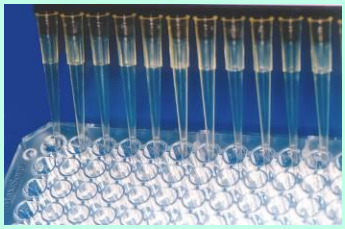
Lighter color = Lower concentration
Darker color = Higher concentration

Items Not Provided

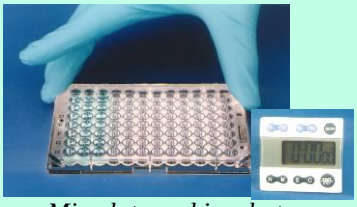
- PBS/0.05% Tween-20 Wash Buffer (may be purchased in 1L dry packets from Sigma Chemicals, Cat#P-3563, or prepared from salts on site). Store at controlled ambient temperature for up to one week, then discard.
- 1 N Hydrochloric acid (HCl) Stop Solution. Prepare by adding 83 mL of concentrated HCl (36.5-38.0%) 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.
- **Positive Control.** It is recommended that the user prepare a known positive control sample to run in each assay.
- distilled or deionized water for preparing Wash and Extraction Buffer
- Tween-20 (Sigma P-1379, or equivalent) for preparation of Extraction Buffer
- glass bottles or flasks plus graduated cylinder with 1 liter capacity for preparation and storage of Wash and Extraction Buffer
- Snap-cap tubes and pestles for extraction of leaf samples (EnviroLogix Cat# ACC 002, 100/package), optional
- disposable tip, adjustable air-displacement pipettes which will measure 50 and 100 microliters (µL), preferably of multi-channel configuration
- marking pen (indelible)
- tape or Parafilm®
- timer
- microtiter plate reader or strip reader
- wash bottle, or microtiter plate or strip washer
- racked dilution tubes for loading samples into the plate with a multi-channel pipette (optional)
- orbital plate shaker (optional)



Leaf punch using microtube cap



Add Extraction Buffer Blank, Negative and Positive Control, and each sample extract to the plate



Mix plate and incubate



Add Enzyme Conjugate

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. If more wash buffer is needed, order item # P-3563 from Sigma Chemical Co. (St. Louis, MO), or prepare the equivalent.

Extraction Buffer: Add 0.5 mL Tween-20 to 100 mL of prepared Wash Buffer, and stir to dissolve. Store refrigerated when not in use; warm 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.

Single Seeds or Leaf Punches:

Individual seeds:

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. Whatever the method used, take extreme care not to cross-contaminate between seed samples.
2. Add 1 mL of Extraction Buffer to each crushed seed. Mix for at least 30 seconds. Allow extracts to settle completely. Dispensing particles into the test plate can cause false positive results.

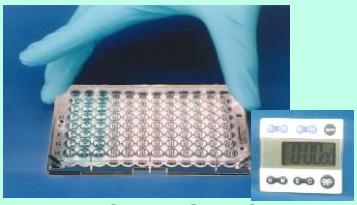
   CAUTION: Vip3A protein is expressed at high concentrations in corn seed, so there is serious potential for cross-contamination between samples during seed crushing. Use the utmost care to avoid this. Cleaning the cutting/crushing surfaces with an alcohol-soaked pad between samples is recommended.

Leaf testing:

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 Extraction Buffer per leaf punch. Mix for at least 30 seconds, and 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.

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 plates from bag with desiccant until they have warmed up).
- Organize all Controls, 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.



Mix plate and incubate



Bottle Wash method



Strip / Plate Wash option



Slap inverted plate on towel to remove as much liquid as possible

- Use the well identification markings on the plate frame as a guide when adding the samples and reagents. In a qualitative assay, the Blank (BL), Positive Control (PC) in duplicate wells, and 92 sample extracts (S) in single wells may be run on one plate. (See the Qualitative Assay Example Plate Layout - Figure 1).

1. Add **50 µL Vip3A-enzyme Conjugate** to each well. Immediately add **50 µL** of **Extraction Buffer Blank (BL)**, **50 µL** of **Positive Control (PC)**, and **50 µL** of each **sample extract and user-prepared control 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 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.

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 mean, blank-subtracted OD of the Positive Control wells should be at least 0.15.



Complete protocol and add Stop Solution



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



- 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 wells. This number is the "Positive Control Ratio".

Interpret the Qualitative Results

Single leaf and seed samples:

If the Positive Control Ratio calculated for a sample is less than 1.0, the sample does not contain Vip3A at the levels normally found in Vip3A-expressing corn.

If the Positive Control Ratio of a sample is greater than or equal to 1.0, the sample is a Vip3A-expressing variety.

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, 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 1. 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 test well strips from one Plate Kit with reagents or test well strips 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 for use 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.



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