VERSION: 1.2	**DATE:** 10/2020
PATHOGEN: Tobacco Ringspot Virus	
HOST: Soybean (Glycine max)	
COMMON NAME:	
METHOD: Sb 5.1 ELISA (formerly Sv 1.1)	
METHOD CLASS: TEMPORARY STANDARD (B)	
SAMPLE: 400 seeds	

REVISION HISTORY: 10/2020 - measurement correction on Step #10.

MATERIALS:

Antiserum: suitable for detection of Tobacco Ringspot Virus (e.g. Agdia, AC Diagnostics, DSMZ, etc.)
Distilled or deionized water
Extraction and ELISA buffers
Microtiter plates: 96 well plates, suitable for ELISA
Grinder: capable of grinding seeds to fine flour
Micropipette
Micropipette tips
Microplate spectrophotometer capable of operation at 405 nm

PROCEDURE:

Preparation of ELISA plate:

1. Dilute Tobacco ringspot virus coating antibody in coating buffer as defined by the supplier.

2. Add 100 µl of coating solution to each well.

3. Cover plates to minimize evaporation.

4. Incubate plates for 4 hours in a humid box or overnight at 4°C.

Extraction of the virus:

1. Grind 4 replicates of 100 soybean seeds, per sample and add 100 ml of General Extraction Buffer.

2. OR, place 4 replicates of 100 soybean seeds into 100ml of General Extraction Buffer; soak overnight at room temperature, then grind.

3. Disinfect the grinder between samples by rinsing with distilled water, followed by a rinse with a laboratory or hospital standard detergent, then a final rinse with distilled water.

Running the ELISA kit:

1. Empty coated plate and wash 3 times with PBS-Tween.

2. Pipette 100 µl of seed extract into appropriate wells.

3. Add positive and negative controls to the plate.

4. Incubate plates for 2 hours in a humid box at room temperature, overnight at 4°C or as defined by the antibody supplier.

5. Prepare enzyme conjugate dilution according to supplier directions, use within 10 minutes of preparation.

6. Remove seed extracts from the plate and wash the plate 3 to 5 times with PBS-T.

7. Add 100 µl of diluted conjugate solution to each well.

8. Incubate for 2 hours at room temperature.

9. Wash plate 6 to 8 times with PBS-T.

10. Prepare PNP substrate solution (10 mg para-nitrophenylphosphate in 10 ml substrate buffer (makes enough for 96 wells, adjust volumes if necessary).

11. Add 100 µl substrate solution to each well.

12. Incubate in the dark for 30 to 60 minutes or as specified by the manufacturer.

Evaluating ELISA plates:

Evaluate results using a spectrophotometer plate reader at 405 nm. The threshold for a positive reaction on the plate reader should be greater than 2X the negative control.

BUFFERS:

General Extraction Buffer (pH 7.4)

Sodium sulfite (anhydrous)	1.3g
Polyvinylpyrrolidone (PVP) mol. wt. 24,000-40,000	20.0g
Sodium azide	0.2 g
Egg albumin (Ovalbumin Grade II)	2.0 g
Tween-20	20.0 g

Dissolve in 1000 ml of 1X PBST.
Store at 4°C.

PBST Buffer (1X, pH 7.4)

Sodium Chloride	8.0 g
Sodium phosphate, dibasic (anhydrous)	1.15 g
Potassium phosphate, monobasic (anhydrous)	0.2 g
Potassium chloride	0.2 g
Tween-20	0.5 g

Dissolve in 1000 ml of distilled water.

Substrate Buffer (pH 9.8)

Diethanolamine	97 ml
Hydrochloric acid (32%)	15 ml

Add water to make to 1 liter.
Adjust pH if necessary with HCl.