Arabis Mosaic Virus (ArMV) - TAS ELISA Kit

Catalog #: V165

Source Antibody: A rabbit polyclonal antibody for capture and and a monoclonal antibody as detection.

Test Format: TAS ELISA

Reactivity: Reaction of the ELISA is relatively strong. Optical Density at 405_{nm} is in a range of 1.400 - 2.600 depending on the virus titer in the samples tested.

Sensitivity: Sensitivity of the ELISA is high. The virus can be consistently detected in infected plant tissues diluted at 1:810.

Specificity: This test system has no cross reaction with healthy plant tissues such as rose and grape. Background is low on all of the negative control wells.

Background Information:

Name: Arabis Mosaic Virus

Acronym: ArMV

Genus: *Nepoviruses*

Transmission: Mechanical

Main host plants and diseases: Celery, cucumber, grape, hops, lettuce, *prunus* spp., raspberry, strawberry, and sugar beet.

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Components:

Catalog	Size	Item											
		Buffers for DAS/TAS, alkaline phosphatase							Coating Antibody	Detecting conjugate, Alkaline Phosphatase (Bottle A and B)	Control		96-wells ELISA Plates
		Coating Buffer, 10×	SB1 Buffer	PBST Buffer	ECB1 Buffer,10×	PNP Buffer, 5×	PNP tablet, 5mg each	Tween-20			Negative	Positive	
M004-1	500	6mL	11.6g	28.7g	6mL	10mL	10	6.5g	—	—	—	—	—
M004-2	1000	12mL	23.2g	57.23g	12mL	20mL	20	13g	—	—	—	—	—
M004-3	5000	60mL	116g	286.15g	60mL	100mL	100	65g	—	—	—	—	—
V165-C1	500	—	—	—	—	—	—	—	0.25mL	—	—	—	—
V165-C2	1000	—	—	—	—	—	—	—	0.5mL	—	—	—	—
V165-C3	5000	—	—	—	—	—	—	—	2×1.25mL	—	—	—	—
V165-D1	500	—	—	—	—	—	—	—	—	0.25mL (A) 0.25mL (B)	—	—	—
V165-D2	1000	—	—	—	—	—	—	—	—	0.5mL (A) 0.5mL (B)	—	—	—
V165-D3	5000	—	—	—	—	—	—	—	—	2×1.25mL (A) 2×1.25mL (B)	—	—	—
V165-K1	500	6mL	11.6g	28.7g	6mL	10mL	10 tablets	6.5g	0.25mL	0.25mL (A) 0.25mL (B)	1	1	5
V165-K2	1000	12mL	23.2g	57.23g	12mL	20mL	20 tablets	13g	0.5 mL	0.5mL (A) 0.5mL (B)	1	1	10
V165-N1	18	—	—	—	—	—	—	—	—	—	1	—	—
V165-P2	18	—	—	—	—	—	—	—	—	—	—	1	—
V165-R1	500	—	—	—	—	—	—	—	0.25mL	0.25mL (A) 0.25mL (B)	—	—	5
V165-R2	1000	—	—	—	—	—	—	—	0.5 mL	0.5mL (A) 0.5mL (B)	—	—	10
V165-R3	5000	—	—	—	—	—	—	—	2×1.25mL	2×1.25mL (A) 2×1.25mL (B)	—	—	50
V165-R4	100	—	—	—	—	—	—	—	0.05mL	0.05mL (A) 0.05mL (B)	—	—	1
Storage		RT	RT	RT	RT	4℃	-20℃	RT	4℃	4℃	-20℃	-20℃	RT

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Safety and Storage:

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Prevent direct skin and eye contact with, or ingestion of, product components. Obtain medical attention in case of accidental ingestion of reagent components. All reagent components should be stored at the recommended temperature to assure their full shelf life.

Store all buffers at room temperature, except for PNP Substrate Buffer at 4 ^{o}C and the PNP tablets at -20 ^{o}C under dark. Store the prepared 1x buffers at 2-6 ^{o}C refrigerator. The 1x buffers can be store up to 3 months.

Test Preparation:

- Check all the components in the ELISA Kit.
- Make sure all laboratory equipment and facilities required for the test are ready.
- Prepare a humid box for incubation steps.
- Make a copy of the attached recording sheet and create a loading diagram by recording the locations of your samples, controls, and other reagents needed.

Buffer Preparation:

- Preparing 1x Buffer from Concentrate:

To prepare the 1x buffer, dilute the concentrated buffers at 1:10 (Coating and Conjugate buffers), and 1:5 (PNP buffer) with D.H_2O. Pour all the content into D.H_2O and stir the diluted buffers for 10-30 minutes for mixing well and dissolving completely. If only part of the concentrated buffer being used, stir or mix the buffer first before pipetting.

- Preparing 1x Buffer from Powder:

To prepare the 1x buffer, dissolve the powder buffer into D.H_2O at the ratio on the table below. For sample buffer, mix the powder with small amount of D.H_2O into a paste (no lumps) before adding more D.H_2O. Stir for 10-30 minutes for dissolving completely, and make up to final volume.

	1×Sample Buffer			1×TBST Buffer		
Buffer Powder	11.6g	23.2g	115.7g	28.7g	57.3g	286.5g
Tween-20	5.0g	10.0g	50.0g	1.5g	3.0g	15.0g
Final Volume	0.5L	1L	5L	3L	6L	30L

Using the buffers: For detail information on using the buffers for preparing reagents, refer to the Test Instructions included in the ELISA Reagents and/or Kits.

Coating Plate with Antibody:

- Lay out all items that will be required for the plate coating step before beginning. Prepare coating antibody in a container made of glass, polyethylene or any material that does not readily bind coating antibody. Coat the plate immediately after preparing the coating antibody. Some coating antibody can be lost if too much time elapses between diluting the coating antibody and coating the plate.
- The volume of coating buffer required depends on the number of test wells used; 100 µl is needed per test well. One way to estimate the volume needed is to prepare 1 ml of coating buffer for each 8-well strip used, or 10 ml for each 96-well plate.
- Dilute the concentrated coating antibody into coating buffer at the dilution given on the label. Mix well. Always prepare coating antibody immediately before use.
- Pipette 100 µl of coating antibody into each well.
- Incubate the plate in a humid box for overnight in the refrigerator (4 °C) or 4 hours at room temperature (21-24 °C).

Samples Preparation:

- Select symptomatic and/or infective tissues for the test. Leaf tissue is often used in ELISA testing. Plant tissues such as stem, sprout, seed, tuber, root and others can also be used.
- Buffer SB1 can be used as extraction buffer for most of the plant samples. However, other buffers are also recommended for some plant species.
- Grind sample with a mortar and pestle, or other grinding device. If you are using a mortar and pestle, wash and rinse it thoroughly between samples.
- If you extract plant sap, dilute the sap into sample extraction buffer at a ratio of 1:10 (sap volume: buffer volume). Or you can grind plant tissue in extraction buffer at a 1:10 ratio (tissue weight: Extraction Buffer volume).

Plate Washing:

- Wash the plate when the incubation is complete. Use a quick flipping motion to empty the wells into a sink or waste container.
- Wash the plate by filling the wells with PBST, then quickly emptying them again. Repeat 4 to 6 times.
- To remove drops of PBST from the wells after washing, hold the frame upside down and tap firmly on a folded paper towel.

Sample Dispensing and Incubation:

- About 100 µl of diluted sample extract is needed per test well. Always have an additional amount to assure easy dispensing. A convenient way to prepare this diluted sample is to measure 100 µl of undiluted sap into a small test tube, then add 1 ml of extraction buffer.
- Following your loading diagram on your recording sheet, dispense 100 µl of prepared sample into sample wells. Dispense 100 µl of positive control into positive control wells, and dispense 100 µl of negative control or extraction buffer into negative control wells.
- Put the plate inside the humid box and incubate for 2.5 hours at room temperature (21-24 °C) or overnight in the refrigerator (4 °C).

Preparing Enzyme Conjugate:

- Always make enzyme conjugate solution within 10 minutes before use. Prepare the enzyme conjugate, using buffer ECB1 and a cleaning container.
- The volume of buffer ECB1 required depends on the number of test wells used; 100 µl are needed per test well. To estimate the volume needed, prepare 1 ml for each 8-well strip used, or 10 ml for each 96-well plate.
- The volume of enzyme conjugate required for each test is calculated based on the volume of buffer ECB1 used and, on the dilutions, given on the bottles. Use a new, sterile pipette tip and change the tip for each pipetting to prevent contamination.
- First dispense appropriate volume of buffer ECB1 into a cleaning container, then add enzyme conjugate from bottle A and bottle B according to the dilution given on the label. For example, if the dilutions given on bottles A and B are both 1:200 and you are preparing 2 ml of enzyme conjugate, you should first dispense 2 ml of ECB1 buffer. Then add 10 µl from bottle A and 10 µl from bottle B to the ECB1 buffer.

- After adding the conjugates from bottles A and B, mix the conjugate solution thoroughly. If you prepare the conjugate in a test tube, invert it several times. If you prepare the conjugate in a beaker, stir the conjugate solution with a glass rod. It is important to mix the enzyme conjugate well for a consistent test result.
- Prepare enzyme conjugate just before use. Keep the prepared enzyme conjugate at a safe place and use it after washing the plate.

Plate Washing:

- Wash the plate 6 to 8 times with PBST as instructed above.

Enzyme Conjugate Incubation:

- Dispense 100 µl of prepared enzyme conjugate per well for all test wells.
- Incubate the plate in the humid box for 2.5 hours at room temperature (21-24 °C).

Preparing Substrate Solution:

- Concentration of PNP in substrate is 1 mg/ml. Each PNP tablet will make 5 ml of PNP solution, which is enough for 48 test wells or five 8-well strips.
- Prepare PNP substrate about 10-15 minutes before the end of the above incubation step. Measure 5 ml of buffer PNP for each tablet, then add the PNP tablets to the buffer. Mix by vortexing or stirring to let the PNP tablet fully dissolve in the buffer.

Plate Washing:

- Wash the plate 6 to 8 times with PBST as instructed above.

Incubation with Substrate:

- Dispense 100 µl of PNP substrate solution per well.
- Incubate the plate for 30 to 60 minutes in a humid box at room temperature (21-24 °C).
- To stop reaction, add 50 µl of 3M sodium hydroxide to each well (optional). The plate can be interpreted visually or with a plate reader without adding the stop solution.

Evaluating Results:

- Test results can be examined by eye, or measured on a plate reader at 405 nm.
- Development of yellow color in test wells indicate positive results. Wells in which there is no significant color development indicate negative results. Test results are valid only if positive control wells give a positive result and negative control wells remain clear.
- Results may be interpreted after more than 60 minutes of incubation as long as negative control wells remain virtually clear.

Notes:

- We suggest that each test well be used for only one sample. In some cases, composites of up to ten leaves per test well can be used to make testing more economical. However, too many plant samples per well can reduce the sensitivity of the test.
- Do not touch the PNP tablets or expose the PNP solution to strong light. Light or contamination could cause background color in negative wells.
- If you have any questions about sampling, sample preparation, or the appropriate extraction buffer for your samples, please contact info@nanodiaincs.com.
- All reagents are recommended for research only.

Buffer Formulation:

Coating Buffer (Adjust pH to 9.6, Store at 4°C)	
Reagent	Quantity
Sodium carbonate (anhydrous)	1.60g
Sodium bicarbonate	2.92g
Sodium azide	0.2g
Distilled water	To 1000mL

PBST Buffer (Adjust pH to 7.3)	
Reagent	Quantity
Sodium phosphate, dibasic, (anhydrous)	1.15g
Potassium phosphate, monobasic (anhydrous)	0.2g
Sodium chloride	8.0g
Potassium chloride	0.2g
Tween-20	0.5g
Distilled water	To 1000mL

SB1 Buffer (Adjust pH to 7.3, Store at 4°C)	
Reagent	Quantity
Powdered egg (chicken) albumin, Grade II	2.0g
Polyvinylpyrrolidone (PVP) MW, 24-40,000	10.0g
Sodium sulfite (anhydrous)	1.3g
Sodium azide	0.2g
Tween-20	10.0g
1X PBST.	To 1000mL

ECB1 Buffer (Adjust pH to 7.3, Store at 4°C)	
Reagent	Quantity
Bovine serum albumin (BSA)	2.0g
Polyvinylpyrrolidone (PVP) MW 24-40,000	10.0g
Sodium azide	0.2g
1X PBST	To 1000mL

PNP Buffer (Adjust pH to 9.8 with hydrochloric acid, Adjust final volume to 1000 mL with distilled water. Store at 4°C)	
Reagent	Quantity
Diethanolamine	97.0mL
Magnesium chloride	0.1g
Sodium azide	0.2g
Distilled water	To 800mL

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RECORDING SHEET FOR ELISA

TEST: ____________________ **DATE:** ______________ **BY:** ____________

TIMING: Coating: __________ **Sample:**__________ **EC:** ________ **Substrate:** _________

KEY POINTS: ____

Coating Antibody: __________ **ul, Coating Buffer:** __________ **ml,**

Enzyme Conjugate: __________ **ul, ECB1:** __________ **ml,**

PNP Substrate: __________ **ml**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

RESULTS/CONCLUSIONS:

1. ____

2. ____

3. ____

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