1.0 Intended Use and Composition

Arsh Biotech Tomato leaf curl New Delhi virus (ToLCNDV) DAS ELISA kit is an immunoassay to detect ToLCNDV in plant samples. This kit is available in 2 formats: For 96 tests (AB-ToLCNDV-01) and for 480 tests (AB-ToLCNDV-05). The kit components are as under:

Description	Part No.	AB ToLCNDV-01 96 Tests	AB ToLCNDV-05 480 Tests
ELISA Solid Plate (uncoated)	AEP01	1	5
ToLCNDV Primary Antibody	TPY01	1	5
Positive Control	TPC01	1	1
Negative Control	TNC01	1	1
ToLCNDV Secondary Antibody	TSY01	1	5
Enzyme Substrate Buffer	TES01	25 ml	125 ml
Substrate Tablets	TST01	5	25
Coating Buffer	ACB01	25 ml	125 ml
1X Wash Buffer	TWB01	1	5
Sample Extraction Buffer	TSD01	1	5
Conjugate Diluent	TCD01	2	10

2.0 General Information

Tomato leaf curl New Delhi virus (ToLCNDV) is a begomovirus of the family Geminivirus, which was first described on tomatoes in India. It is a bipartite virus containing DNA-A and DNA-B as its genome, which is transmitted by whitefly (Bemisia tabaci) and is known to infect a large number of host plants. While ToLCNDV was initially found to infect Solanum lycopersicum (tomato), it was subsequently discovered to also infect other Solanaceae such as Solanum tuberosum (potato) and chili pepper (Capsicum spp.), as well as many Cucurbitaceae, such as: Benincasa hispida (wax gourd), Citrullus lanatus (watermelon), Cucumis melo (melon), Cucumis sativus (cucumber), Cucurbita moschata (musky gourd) and Cucurbita pepo (pumpkin).

The devastating effect of ToLCNDV is common all over the world especially in the tropics and sub-tropics, which are its major infection zone. The disease has been reported from various parts of Asia including India, Bangladesh, Indonesia, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand, and has also been reported in Spain and Tunisia.

Potato Apical Leaf Curl Disease

A severe leaf curl disease has been observed since 1999 in potato (Solanum tuberosum) crops in northern India. The affected plants are severely stunted with apical leaf curl and crinkled leaves, and present a conspicuous mosaic. Diseases caused by ToLCNDV on its different host plants generally include yellow mosaic, leaf curling, vein swelling, and plant stunting. On fruiting crops, when the virus infection occurs at an early stage, affected plants are severely stunted and fruit production is significantly affected, if not supressed.

Arsh Biotech ToLCNDV ELISA kit is designed for detection and identification of ToLCNDV in a standard double antibody sandwich (DAS) ELISA format.

3.0 Principle of the Test

Virus detection is based on a simple double antibody sandwich ELISA. DAS ELISA uses antibodies which are bound to the surface of a microtiter plate to capture the antigen of interest. A specific antibody-enzyme conjugate is then used to detect the trapped antigen. The presence of enzyme is detected by a colorimetric substrate reaction. During the first step of the assay the surface of a microliter plate is coated with the antigen-specific coating-antibody. When an antigen-containing sample is added during the second step, the antigen binds to the immobilized antibody, forming an antibody-antigen complex. This complex reacts with the enzyme-labelled antibody-conjugate during the third step by forming a double-antibody sandwich. During the fourth step the enzyme reacts with the substrate in an enzymatic reaction, resulting in yellow coloured product. This colour development can be evaluated visually or measured in a spectrophotometer at 405 nm after 1-2 hours.

4.0 Storage and Stability

Kit components are stable at ambient temperature. Upon receipt, store at 4°C, Positive and Negative controls store at -20°C or below until use. If unopened, the kit is stable until 6 months from the date of manufacture. Expiration date is printed on the kit label.

5.0 Preparation of Reagents

5.1 Ready for use: Store as indicated on labels.

Components	Store at
Coating Buffer	4°C
Enzyme Substrate Buffer	4°C

5.2 To be reconstituted: Store as indicated on labels.

Component	Preparation Instructions
Sample Extraction Buffer	Reconstitute the entire contents in 500 ml deionized water/double distilled water (ddw) and maintain pH 8.5.
Wash Buffer	Reconstitute the entire contents in 500 ml deionized water/ ddw to make 1X wash buffer.
Conjugate Diluent	Reconstitute the entire contents in 50 ml 1X wash buffer to make 1X conjugate diluent.
Substrate	Add provided substrate tablets in provided substrate buffer (4 tablets in 20 ml enzyme substrate buffer).

5.3 To be diluted: As indicated on labels.

Component	Preparation Instructions
Positive Control	Add 2.0 ml of sample extraction buffer to get optimum dilution.
Negative Control	Add 2.0 ml of sample extraction buffer to get optimum dilution.
Primary Antibody	Dilute in provided coating buffer as per recommended dilution (dilution written on the label).
Secondary Antibody	Dilute in provided conjugate diluent as per recommended dilutions (dilution written on the label).

Positive and Negative controls should be aliquot and stored properly (at -20°C or below) for further use.

6.0 Materials required but not supplied

Description	Cat No.
Microplate reader to read wells at 450-620 nm wavelength.	ABMR01
Automatic microplate washer to wash wells.	ABMW01
Pipettes that dispense 20-200µl and 100-1000 µl.	ABMP200 ABMP1000
Plants sample preparation assembly (mortar - pestle/ mesh begs)	ABMP01
Store bottle to store diluted/ reconstituted reagents.	ABOSB5
Sterile deionized water to reconstitute the reagents	ABOW500

7.0 Assay Procedure

7.1 Coating

Coat 96-well plate with the diluted coating antibody at 200µl per well.

⬇

Tap the plate gently from all sides to ensure that there is no bubble or uncovered space and incubate at 37°C for 2 hrs.

⬇

Remove the plate from the incubator and wash three times with 250µl of wash buffer with three minutes soak time between washes.

7.2 Sample Preparation and Loading

Extract sample 1:20 (w/v) in sample extraction buffer. Add 200 µl of clear supernatant of the test sample, 200 µl reconstituted positive and negative controls in the respective wells. For background control add 200µl of sample extraction buffer.

⬇

Cover the plate and incubate overnight at 4°C.

⬇

Wash three times with 250µl of wash buffer with three minutes soak time between washes.

7.3 Detection with Conjugated secondary antibody

Dispense 1X secondary antibody (as prepared in section 5.2) at 200 µl per well and incubate at 37°C for 3 hrs.

⬇

Wash three times with 250 µl of wash buffer with three minutes soak time between washes.

⬇

Transfer freshly prepared enzyme substrate at 200µl per well and incubate at 37°C for 60 minutes.

7.4 Measurement/Visualization

Read absorbance by using microplate reader at 405 nm (with 620 nm reference). Alternatively examine the wells by eye.

8.0 Interpretation

Wells in which colour develops indicate positive result. Wells in which there is no significant colour development indicate negative result. There should not be significant colour reaction in Background control. No significant colour in background control and negative control is an important validity criteria of the assay.

Example OD values for
Positive Control are: 2.109, 2.212
Negative Control are: 0.173, 0.179
Background Control are: 0.182, 0.173

A test sample may be considered positive for the assay if the OD values for the test samples are greater than 3 times the Negative Control OD values in the said assay.

9.0 Sample Plate Set-Up

Recommended Plate Layout for 96 Test Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	BC	BC	6	6	14	14	22	22	30	30	38	38
B	NC	NC	7	7	15	15	23	23	31	31	39	39
C	PC	PC	8	8	16	16	24	24	32	32	40	40
D	TS1	TS1	9	9	17	17	25	25	33	33	41	41
E	2	2	10	10	18	18	26	26	34	34	42	42
F	3	3	11	11	19	19	27	27	35	35	43	43
G	4	4	12	12	20	20	28	28	36	36	44	44
H	5	5	13	13	21	21	29	29	37	37	45	45

Abbreviations: TS= Test Sample,
PC= Positive Control, NC= Negative Control,
BC= Background Control.

N.B Tests are recommended to be performed in duplicates, both for samples and controls.

Technical Support available

Toll Free at 1800-3000-8822

10.0 Common Troubleshooting Tips

Problem	Possible Cause	Solution
No signal or weak signal	Exclusion of key reagents	Check that all reagents have been added in the correct order.
	Old Enzyme Conjugate	Prepare AB-Enzyme Conjugate fresh and do not store it more than 24 hours.
	Enzyme inhibitor present	Contaminants such as Sodium azide inhibit enzyme reactions. Ensure a clean working area and use clean tips.
	Incorrect assay temperature (too cold)	Use recommended incubation temperature. Bring Enzyme Substrate to room temperature before use.
High Background	Poor Washing	Ensure all wells are filled with wash buffer and are being aspirated completely. Use an automated plate washer if available.
	Enzyme Conjugate too high	Use recommended dilutions of reagents.
	Contaminating enzymes present in diluent	Test diluent with substrate alone to check for contaminating enzyme activity.
Uneven colour development or some colour in healthy samples	Incomplete washing of wells	Ensure all wells are filled evenly with wash buffer and are being aspirated completely.
	Error in substrate preparation	Use recommended concentration of enzyme and conjugate. Always use freshly prepared substrate (prepare substrate 15 minutes before use).

Complete troubleshooting guide available at www.arshbiotech.com

11.0 Potato pathogen testing portfolio

Test pathogen	Pathogen Type	Cat No.
Potato Virus A	Virus	AB5305
Potato Virus M	Virus	AB5306
Potato Virus S	Virus	AB5307
Potato Virus T	Virus	AB5308
Potato Virus V	Virus	AB5309
Potato Virus X	Virus	AB5310
Potato Virus Y	Virus	AB5312
Potato virus Y – necrotic strain (PVY$^{n/ntn}$)	Virus	AB5311
Potyvirus Group Test	Virus	AB5315
Tomato Leaf Curl New Delhi Virus	Virus	AB5420
Tobacco Rattle virus	Virus	AB5408
Tobacco Necrosis Virus (A or D)	Virus	AB5400
Tomato Spotted Wilt Virus	Virus	AB5424
Potato Aucuba Mosaic Virus	Virus	AB5295
Potato Leafroll Virus	Virus	AB5303
Potato Spindle Tuber Viroid	Virus	AB5571
Tobacco Mosaic Virus	Virus	AB5393
Phytophthora spp. (Phyt) (Damping off)	Fungi	AB5287
Pythium spp (Pyth)	Fungi	AB5329
Rhizoctonia solani	Fungi	AB5341
Pectobacterium Aatrosepticum (Patro)	Bacteria	AB5266
Erwinia amylovota (Ea)	Bacteria	AB5174
Ralstonia solonaceraum (Rs)	Bacteria	AB5332
Calvibacter Michiganensis Sub sp. Michiganensis	Bacteria	AB5119

CE Certified ISO 9001 ISO 13485
ISO 14001 OHSAS 18001

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ToLCNDV DAS ELISA KIT

To Detect Tomato Leaf Curl New Delhi Virus in Plant Samples

INSTRUCTION MANUAL

FOR ELISA KIT #AB-ToLCNDV-96 (96 Tests)
AND #AB-ToLCNDV-480 (480 Tests)

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ISO 14001 OHSAS 18001

Version 1.05

Technical Support available

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