Leibniz-Institut
DSMZ-Deutsche Sammlung von
Mikroorganismen und Zellkulturen GmbH

Data Sheet on
Tomato leaf curl New Delhi virus (ToLCNDV)

***Tomato leaf curl New Delhi virus* (*Geminiviridae*) is an important pathogen that causes severe crop losses in cucurbitaceous crops and affects tomato (*Solanum lycopersicum*) plants.**

Symptoms caused by ToLCNDV on cucumber and tomato plants

ToLCNDV is known to infect tomato in India since twenty years and its host range and geographic distribution has been expanded since that time. The virus is naturally transmitted by the whitefly species *Bemisia tabaci* and has been shown to be experimentally transmitted by mechanical inoculation. It has been found in several countries in Asia, also infecting cucumber and luffa, and has first been reported in Europe in 2012, infecting zucchini crops in Murcia Province (southern Spain). In 2013, ToLCNDV symptoms were also observed on tomato in Almeria (Spain). Subsequently, it has been shown that the virus can be transmitted to and from zucchini squash. It can be predicted that the virus will spread further in southern Europe and potentially wherever its vector *Bemisia tabaci* persists.

Virus diagnosis:
ELISA is the most reliable assay for the fast routine detection of ToLCNDV in its hosts. Specific polyclonal antibodies and controls developed at the DSMZ Plant Virus Department allow a sensitive and reliable detection and identification of ToLCNDV in a standard DAS-ELISA, offered as certified reference materials according to our ISO Guide 34:2009 and DIN EN ISO 17025:2005 accreditation.

DAkkS Deutsche Akkreditierungsstelle D-RM-18324-01-00

Diagnostic products abvailable:

- ToLCNDV DAS-ELISA set AS-1109
- ToLCNDV positive control PC-1109

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Double Antibody Sandwich ELISA (DAS-ELISA), ToLCNDV

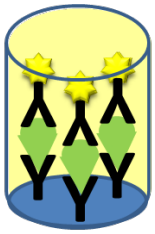
Sampling: For a reliable ToLCNDV detection, it is strongly recommended to sample youngest leaves!

Our ELISA reagents are optimized using greiner bio-one microplates, medium binding.
Before opening the tubes containing coating antibody (IgG) and IgG-AP- conjugate please spin down all the liquid by a short centrifugation (approx. 3000rpm for a few seconds).

1. Dilute **AS-1109, IgG** in coating buffer (recommended dilution see delivery note and tube); i.e. 20µl in 20 ml buffer at a recommended dilution of 1:1000. Add 200µl to each well of the microtiter plate.
2. Cover the plates and incubate at 37 °C for 2- 4 h.
3. Wash plate with PBS-Tween using wash bottle, soak for a few minutes and repeat washing two times. Blot plates by tapping upside down on tissue paper.

4. Extract samples 1/20 (w/v) in **extraction buffer for Begomoviruses (0.05 M Tris containing 0.06 M sodium sulfite, pH 8.5)**. Add 200 µl aliquots of the test sample to duplicate wells.
5. Cover the plates and incubate overnight at 4 °C.
6. Wash three times as in step 3.

7. Add 200 µl enzyme conjugate **AS-1109, IgG-AP**, recommended dilution is given in the delivery note, in conjugate buffer; i.e. 40µl in 20 ml buffer at a recommended dilution of 1:500.
8. Cover the plates and incubate at 37 °C for 2- 4 hours.
9. Wash three times as in step 3.



10. Add 200 µl aliquots of freshly prepared substrate (1 mg /ml para- nitrophenyl- phosphate in substrate buffer) to each well.
11. Cover the plate and incubate at 37°C for 30-60 min, or as long as necessary to obtain clear reactions.
12. Assess results by:
    a) Visual observation
    b) Spectrophotometric measurement of absorbance at 405 nm

Reference: Clark, M. F. and Adams. A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 34: 475-483

Buffers used in ELISA

1. ***Coating buffer (pH 9.6)***

   1.59 g sodium carbonate (Na_2CO_3)
   2.93 g sodium bicarbonate ($NaHCO_3$)
   0.20 g sodium azide (NaN_3)
   Dissolve in 900 ml H_2O, adjust pH to 9.6 with HCl and make up to 1 l.

2. ***PBS (pH 7.4) phosphate buffered saline***

   8.0 g sodium chloride (NaCl)
   0.2 g monobasic potassium phosphate (KH_2PO_4)
   1.15 g dibasic sodium phosphate (Na_2HPO_4)
   0.2 g potassium chloride (KCl)
   0.2 g sodium azide (NaN_3)
   Dissolve in 900 ml H_2O, adjust pH to 7.4 with NaOH or HCl and make up to 1 l.

3. ***PBS-Tween (PBST)***

   PBS + 0.5 ml Tween 20 per liter

4. ***Sample extraction buffer (pH 8.5) for Begomoviruses***

   0.05 M Tris containing 0.06 M sodium sulfite, pH 8.5

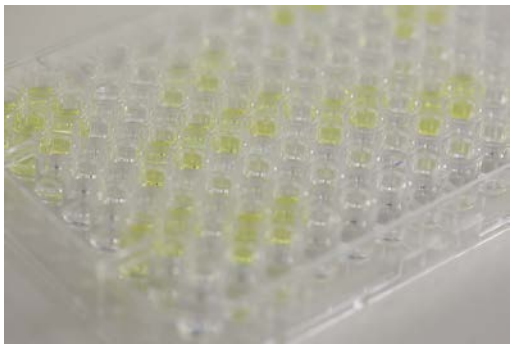
5. ***Conjugate buffer***

   PBST + 2% PVP + 0.2% egg albumin (e.g. Sigma A-5253)

6. ***Substrate buffer***

   97 ml diethanolamine
   600 ml H_2O
   0.2 g sodium azide (NaN_3)
   Adjust to pH 9.8 with HCl and make up to 1 liter with H_2O

Buffers can be stored at 4 ° C for at least 2 months. Warm buffers to room temperature before use.



ELISA Troubleshooting

1. *No color development*

a) Did you omit any steps?
b) Did you use correct buffer for each step?
c) Is your enzyme OK? Serum OK?
d) Is your positive control homologous to antiserum (IgG)?

Recommendations - Do a titration plate. Use reliable positive control in each plate. Pretest enzyme conjugate on substrate.

2. *Nonspecific color development*

a) If in edge wells only:
- Make sure the humidity in the incubator is sufficiently high.
- If this does not help, don't use edge or border wells, fill with buffer only.

b) If in whole plate:
- incomplete washing
- old substrate
- use recommended ELISA plate (greiner medium binding)
- error in loading sequence

Recommendations - Use reliable negative control in each plate. Use fresh substrate and check for spontaneous colour change. Cover plates. Check pH of the buffers used.

c) Some wells with inconsistent or unexpected reactions
- incomplete washing
- error in loading test antigens
- spillage between wells

Recommendations - Use extra wash step, handle plates carefully with lids on, use predetermined loading pattern before loading. Blot top of plate after rinsing.

3. *Color development very rapid; some color in healthy samples*

a) Enzyme conjugate concentration too high
b) Substrate concentration too high

Recommendations - Use enzyme conjugate and substrate concentrations that will give $OD_{405\ nm}$ of about 1.0 in 30 to 60 min with good antigen source.

Virus isolates and serological tests are available at the DSMZ Plant Virus Department and can be ordered via web shop (www.dsmz.de), email (plantvirus@dsmz.de) or by fax (+49 -511 -2616 455).