Product Information: PTA-ELISA

Potygroup test (Poty group)

The potyvirus group test (1,2) recognizes members of the genus *Potyvirus*. This genus is the largest plant virus group recognized by ICTV, consisting of many economically important virus species that cause diseases in potatoes, vegetables, ornamentals, fruit and field crops etc. These viruses are transmitted by aphids. The poty group reagents are used in an indirect PTA-ELISA format where the plant samples (extracted in a special buffer) are directly coated to the microtiter plate (procedure described below). The antibodies are available only in complete kits, containing all reagents, controls, microtiter plates, buffers and substrate necessary to perform the tests (for more detailed information, see next page).

Specificity and sampling instruction

The antibodies are monoclonal (P-3-3H8) and were raised against the peanut stripe virus isolate of Bean common mosaic virus (3, and H.J. Vetten, personal communication). They have been shown to react with a large number of viruses of the genus *Potyvirus* and also with some members of the genus *Rymovirus* (3). However, they did not react with members of the genera *Bymovirus*, *Ipomovirus* and *Tritimovirus* (3). Homogenize a plant sample of approx. 0.1 g in 5 ml (1:50 w/v) special extraction buffer «PTA» (3). With certain samples (e.g. tobacco), further dilution to 1:500 (w/v) in extraction buffer gives even stronger results. On the other hand, more concentrated extracts (1:10-1:20, w/v) generally give weaker results.

The product is based on antibodies developed by the Julius Kühn-Institut (JKI) Braunschweig, Germany (Bundesforschungsinstitut für Kulturpflanzen; Institut für Epidemiologie & Pathogendiagnostik).

Information on the antibodies

Probe IgG: monoclonal

References

(1) Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L. and Zurcher, E.J. (eds.) (1996 onwards). Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996.
(2) Hollings, M., and Brunt, A.A. 1981. Descriptions of Plant Viruses. No. 245. CMI/AAB. 7 pp.
(3) Richter, J., F. Rabenstein, E. Proll & H.J. Vetten (1995). Use of cross-reactive antibodies to detect members of the Potyviridae. J. Phytopathology 143, 459-464.

Test procedure

1) Homogenize plant samples at ratios of 1:50 (w/v) with the freshly diluted extraction buffer «PTA» (see page 2/2). Resuspend the lyophilized controls in 2.5 ml of the same extraction buffer. Distribute the extracts and controls in portions of 200 µl into the wells of a microtiter plate included in the Complete kit. Duplicate wells per test sample increases test security. Cover the plate with parafilm, rubber sheet, and cover, and incubate overnight at 4°C.
2) Wash the plate carefully 3-4 times with PBST. Do not let the plate stand with empty wells at any time!
3) Dilute the appropriate portion of the IgG 1:1000 in conjugate buffer (e.g. for one plate: 20 µl in 20 ml conjugate buffer) and dispense 200 µl per well. Cover the plate as described above and incubate for 2 h at 37°C.
4) Wash 3-4 times with PBST.
5) Dilute the goat anti mouse AP conjugate 1:1000 in conjugate buffer and dispense 200 µl per well. Cover the plate as described above and incubate for 2 h at 37°C.
6) Wash 3-4 times with PBST.
7) Add 200 µl pNPP solution* per well and incubate for 60-120 min at room temperature.
8) Read results at 405 nm (if available, read with a reference filter at 405/492 nm).

* to obtain the pNPP solution, add 1 pNPP tablet of 20 mg to 20 ml of substrate buffer 15 min before use.

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Kit Content (for 480/960 assays)

Extraction Buffer «PTA»: 250 ml/500 ml concentrate (10x). Before use, make up with double distilled water to 2500 ml/5000 ml or prepare smaller portions at equal ratio. Once diluted, this buffer is stable for up to two months at 4°C. The use of this buffer is essential. Other extraction buffers as used for DAS-ELISA do not work!

Washing Buffer: 5/10 tablets, each dissolved in 1000 ml of double distilled water, give phosphate buffered saline with 0.05% Tween 20 (PBST). This solution contains no NaN3! Use within 2 days or add a preservative (NaN3 at 0.2 g/l) to prevent microbial growth!

Conjugate Buffer: 20 ml/2 x 20 ml concentrate (10x). Before use, make up with double distilled water to 200 ml/ 2 x 200 ml. Once diluted, this buffer is stable for up to two months at 4°C.

Anti Poty group IgG: One vial containing 0.1 ml/0.2 ml of monoclonal antibody (IgG from mouse) specific to members of the genus Potyvirus. Before use, dilute 1:1000 in conjugate buffer. Spin down drops of the IgG concentrate, which might stick to the lid with a microcentrifuge (approx. 3000 RPM for a few seconds)!

Goat anti mouse IgG AP conjugate: One vial containing 0.1 ml/0.2 ml of polyclonal antibody labeled with alkaline phosphatase. Before use, dilute 1:1000 in conjugate buffer. Spin down drops of the conjugate concentrate, which might stick to the lid (approx. 3000 RPM for a few seconds)!

Microtiter plates: We strongly recommend to use the included high binding microtiter plates.

Substrate Buffer: 1 x 20 ml/2 x 20 ml substrate buffer (5x). Before use, make up with double distilled water to 100 ml/2 x 100 ml. Add one pNPP tablet (20 mg) per 20 ml buffer 15 min before use.

Substrate (pNPP tablets): 5/10 single packed tablets containing each 20 mg of pNPP (p-nitro-phenyl-phosphate). Open the blister pack without touching the content and add one tablet per 20 ml of substrate buffer to obtain a solution of 1 mg/ml.

Controls: One vial each of positive and negative control. Just before use, reconstitute the lyophilized content in 2.5 ml of extraction buffer «PTA». The unused portion can be divided into aliquots and frozen at -20°C or colder for later use. Avoid repeated freezing and thawing!