Product Information: DAS-ELISA

Potato virus Y (PVY)

Introduction to potato virus Y (PVY)

For PVY in potato, several strain groups are distinguished according to the symptoms on *Nicotiana tabacum*, *Physalis floridana*, *Solanum tuberosum* and other potato cultivars (4). The main groups are: the common (PVY^{O}), tobacco veinal necrosis (PVY^{N}), and stipple streak (PVY^{C}) strain groups of PVY. More recently, subgroups within the historical groups have been described. For example within the PVY^{O} group, atypical isolates, originating mostly from pepper and tobacco, have been assigned to the subgroup PVY^{Ob}, whereas the common European potato isolates are called PVY^{Oa} (8). Within PVY^{N}, subgroups are represented by more recently described isolates such as PVY^{NTN} (involved in the potato tuber necrotic ringspot disease) (9) and the Wilga type $PVY^{N}W$ (2). The latter one ($PVY^{N}W$) is biologically similar to the «old» N-type (inducing typical necrosis on tobacco), but serologically similar to the O-type (no reaction with our «PVY-necrotic» reagent). Details of detection of these isolates with our PVY reagents are given below in the section specificity and are illustrated in Table 1.

BIOREBA AG has collaborated since 1980 with Agroscope, the Swiss centre of excellence for research in the agriculture and food sector, and other institutes worldwide for the development of ELISA reagents (7, 8). These collaborations allow us to adjust our reagents to new knowledge and new findings as needed. In this regard, we, in cooperation with Agroscope, were able to modify and thus optimize our full-spectrum PVY reagent («PVY monoclonal cocktail») introduced many years ago (for details see below).

We rely as much as possible on monoclonal antibodies (Mab) for our PVY ELISA reagents for the following reasons:

- The reagent based on Mab guarantees a well defined and standardized product (constant in specificity and avidity). Once developed, Mab are reproducible year after year.
- Different complementary Mab can be mixed in a controlled manner in order to broaden its serological range as it has been done for example with our «PVY monoclonal cocktail». Furthermore, Mab are suitable for differentiating PVY^{N} subgroups (example described in section D below).
- Pathogen-specific Mab do not contain antibodies against host plant proteins that might interfere in the test. Therefore, non-specific background in ELISA is generally decreased, rendering the test more sensitive (wider OD ratio between infected and healthy tissue). There are exceptional cases where non-specific reactions with Mab do occur (non-specific reactions with certain plant components?).

Our ELISA reagents have been extensively tested in Europe and at the International Potato Center (CIP) in Peru and were found to be very efficient (W. Bitterlin and P. Gugerli, unpublished; COST 88 PVY ring-test, unpublished; 5,7,10). Furthermore, they have been used since the early 1980's by many scientists and in numerous potato certification laboratories in many parts of the world.

Specificity of reagents in DAS-ELISA (see also Table 1)

A. The traditional broad-spectrum PVY reagent («PVY monoclonal»), made against isolate PVY^{N}605 and on the market since 1982, recognizes isolates belonging to the tobacco veinal necrosis (PVY^{N}), common (PVY^{O}) and stipple streak (PVY^{C}) strain groups of PVY (7). The reagent is based on a monoclonal antibody (Mab) to a common antigenic determinant. All of today's known PVY isolates are recognized by this Mab with the exception of a few atypical isolates (e.g. PVY^{O}768) belonging to subgroup PVY^{O}b (8). This reagent also recognizes PVY^{NTN}(involved in the potato tuber necrotic ringspot disease) (9) and the Wilga type $PVY^{N}W$ (2). The reagent has proven its validity over the past quarter of a century in variable testing set-ups all over the world. As the first monoclonal-based reagent that became commercially available for phytodiagnostics, it really has become a true PVY standard reagent.

Information on the antibodies

Coating IgG: monoclonal; conjugate: monoclonal

www.bioreba.com

BIOREBA AG — *Your Partner in Agro-Diagnostics*
Christoph Merian-Ring 7
CH-4153 Reinach BL1
Switzerland
phone +41 61 712 11 25
fax +41 61 712 11 17
admin@bioreba.ch
www.bioreba.com

B. The full-spectrum PVY reagent («PVY monoclonal cocktail») contains complementary monoclonal antibodies to isolates from different strain groups (7, 8). It recognizes the whole range of PVY isolates from the tobacco veinal necrosis (PVY^{N}), common (PVY^{O}) and stipple streak (PVY^{C}) strain groups of PVY (Table 1). All recently described isolates such as PVY^{O}768 (belonging to subgroup PVY^{Ob}) (8) or PVY^{NTN} (involved in the potato tuber necrotic ringspot disease) (9) and the Wilga type PVY^{N}W (2) are recognized. This reagent generally gives a more uniform reaction with the virus and a lower background than polyclonal antibodies from rabbit or sheep serum. The current reagent that became available in 2002 is an improved version of the reagent introduced in 1994. It consists of a «cocktail» of different complementary monoclonal antibodies. No cross-reaction with any other known potato virus has been observed.

Information on the antibodies

Coating IgG: monoclonal; conjugate: monoclonal

C. The polyclonal PVY reagent («PVY polyclonal») was made against isolate PVY^{N}605. It recognizes, similar to «PVY monoclonal cocktail», the whole range of isolates from the tobacco veinal necrosis (PVY^{N}), common (PVY^{O}) and stipple streak (PVY^{C}) strain groups of PVY, including the more recently described isolates such as PVY^{O}768 (belonging to subgroup PVY^{Ob}) (8) or PVY^{NTN} (involved in the potato tuber necrotic ringspot disease) (9) and the Wilga type PVY^{N}W (2).

Information on the antibodies

Coating IgG: polyclonal; conjugate: polyclonal

D. The necrotic strain group-specific PVY reagent («PVY necrotic») specifically recognizes isolates belonging to the tobacco veinal necrosis (PVY^{N}) strain group of PVY («old» N-type group), including subgroup of PVY^{NTN} (involved in the potato tuber necrotic ringspot disease) (9). In an independent study, carried out in 1991 (COST 88 PVY ring-test, *unpublished*), where nearly 50 PVY isolates from Europe and other continents were compared in biological and serological tests, all isolates of PVY^{N} (old N-type group) were recognized with our PVY^{N} reagent. On the other hand, Wilga type PVY^{N}W isolates (2) are not recognized (PVY^{N}W isolates are recognized with our other PVY reagents, i.e. «PVY monoclonal», «PVY monoclonal cocktail», and «PVY polyclonal» described in sections A,B and C). Our PVY reagents thus provide a suitable tool for differentiating PVY^{N} subgroups (M. Chrzanowska, personal communication, and 2). The PVY^{N} reagent is based on monoclonal antibodies, developed against PVY^{N}605 (7,8). The coating reagent consists of broad-spectrum, the conjugate of N-specific Mab (7). The PVY^{N} reagent has been developed for double antibody sandwich ELISA (DAS-ELISA) (3,6). This format is essential to ensure the described N-specificity.

Information on the antibodies

Coating IgG: monoclonal; conjugate: monoclonal

Sampling instruction

For all potato reagents, we recommend our extraction buffer «General» (Art. No. 110120) for testing leaf samples at a ratio of 1:20 (w/v). For testing sprouted tuber samples (sprouts of potato), a special extraction buffer «Bulbs & Tubers» (Art. No. 110122), containing egg albumin (ovalbumin), is used at a ratio of 1:10 to 1:20 (w/v; 7).

This product has been developed in cooperation with Agroscope, the Swiss centre of excellence for research in the agriculture and food sector.

Table 1.
Reaction of isolates of PVY with different reagents in DAS-ELISA

Virus isolate[a]	DAS-ELISA reagents A PVY monoclonal	B PVY monoclonal cocktail	C PVY polyclonal	D PVY^N (necrotic) monoclonal
PVY^N 605 representing „old" PVY^N group	+++[b]	+++	++	+++
PVY^{NTN} 59 representing PVY^{NTN} subgroup	+++	++	++	+++
PVY^N Wi-1984 representing PVY^N W subgroup	+++	++	+	-
PVY^O 803 representing PVY^{Oa} subgroup	+++	+++	++	-
PVY^O 768 representing PVY^{Ob} subgroup	-	++	+	-
PVY^C 632 representing PVY^C group	+++	++	++	-
Healthy potato leaf[c]	-	-	-	-
Healthy potato tuber[c]	-	-	-	-

[a] The virus isolates are representative for different strain groups of PVY.
[b] +++ = very strong reaction, ++ = strong reaction, + = clearly positive reaction, - = negative reaction.

References

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Ordering Information

BIOREBA offers the following formats:

Individual ELISA reagents for 96, 480, 960 or 5000 assays: IgG and/or conjugate for the working volume of 200 µl/test/well.

Reagent sets for 480, 960 or 5000 assays: IgG and conjugate, positive and negative controls, and micro-titer plates (F-96) for a working volume of 200 µl/test/well.

Complete kits for 96, 480, 960 or 5000 assays: All reagents, controls, microtiter plates (F-96), buffers, and substrate necessary for a working volume of 200 µl/test/well.

ELISA buffers, equipment for sample preparation and disposables are also available.

For all Art. No. please refer to our product catalogue or our homepage www.bioreba.com and for prices and further information on any other product from BIOREBA, please contact your local distributor or our office in Switzerland.