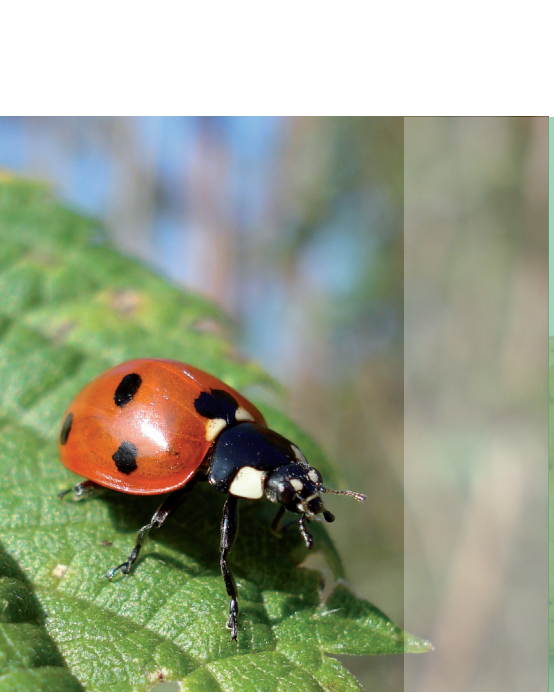
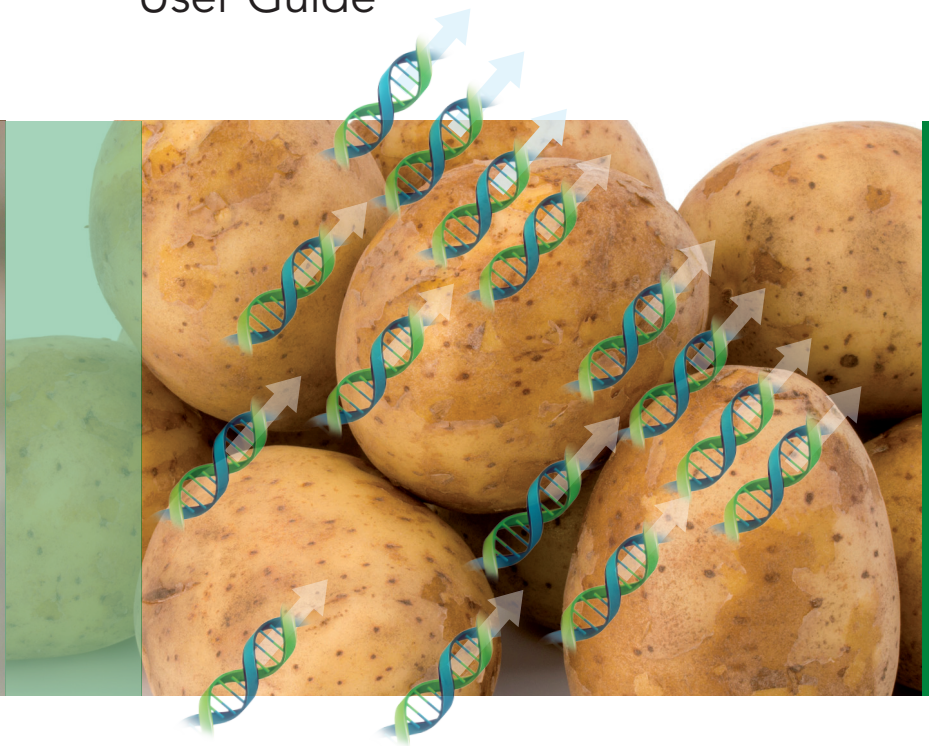
BIOREBA

PCR macroarray potato virus kit

Diagnostic kit for multiplex detection of potato viruses PVA, PVM, PVS, PVX, PVY (O- and N-type), PLRV, PMTV and PSTVd

User Guide





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1. Introduction

1.1 General

The PCR macroarray is a diagnostic method for the detection of eight potato pathogens (7 viruses and 1 viroid) in one single reaction. The following viruses can be detected: PVA, PVM, PVS, PVX, PVY (O- and N-type), PLRV, PMTV and PSTVd. The principle is to amplify viral RNA, extracted from potato tuber or leaf by RT-PCR. Specific probes immobilized on the array will bind the amplified viral cDNAs, which are labeled with biotin. Alkaline phosphatase (APase) conjugated with streptavidin then binds to the biotin of the bound, positive samples. Positive samples are visualized by the colorimetric reaction of the APase with the substrate NBT / BCIP (see Figure 1).
The sensitivity of the PCR macroarray is higher than that of ELISA or traditional RT-PCR. To perform a test, less than 5 hours are required.

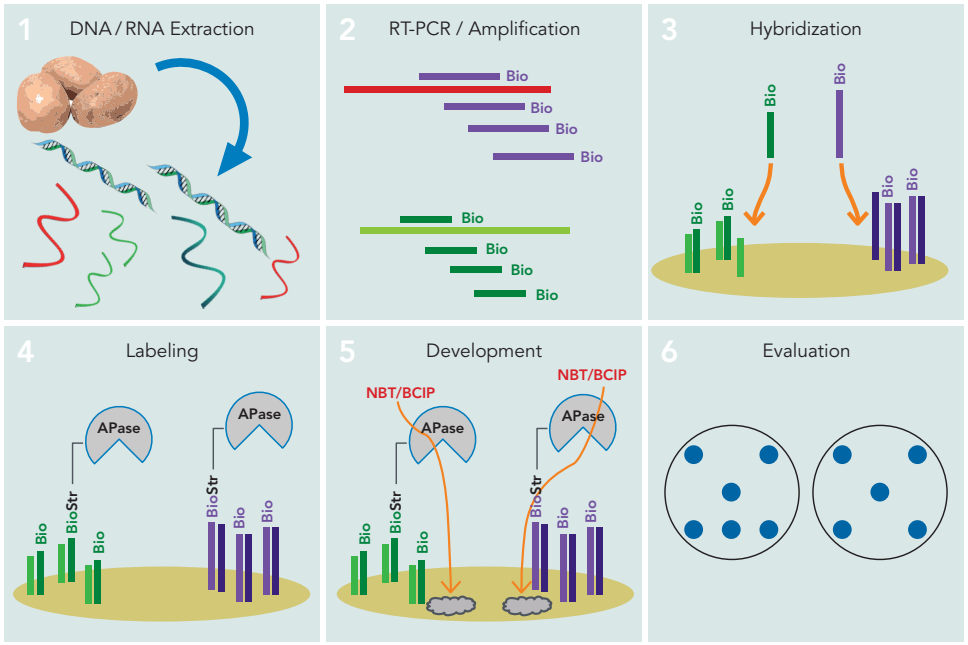


Figure 1: The six main working steps of the potato PCR macroarray

Nucleic acid extraction (1), amplification of pathogen RNAs with specific primers (labeled with biotin) (2), hybridization of the amplified cDNAs on the macroarray (3), labeling of the positive samples with alkaline phosphatase (conjugated with streptavidin) (4), colorimetric reaction with NBT/BCIP as substrate (5) and evaluation of the results (6).

1.2 Special handling instructions

Perform RNA extractions in an RNase-free work environment. Always wear gloves when handling samples containing RNA and kit components. Do not pick up any kit component with an ungloved hand. Keep all kit components tightly sealed when not in use. Keep all tubes containing RNA tightly sealed during the incubation steps. Use appropriate laboratory consumable parts. Especially use nuclease free tubes and filter tips to avoid degradation and cross contamination. Do not use kit components with different lot numbers together. In order to avoid cross-contamination and receive reliable results, it is essential to strictly follow the protocol in this manual. Each working step, extraction of RNA, preparation of the PCR reactions and the amplification/hybridization should be performed in different rooms to prevent cross-contamination.

1.3 Warranty and liability

BIOREBA products are guaranteed to meet the specifications described on the product certificate of analysis and user guide that is added to each shipment.
No further warranties are given. If you have any questions about specification or performance, please contact our administration (admin@bioreba.ch). Our products are for laboratory use only and are not to be used for human or animal applications. Should a product fail for reasons other than inappropriate handling or misuse, BIOREBA AG will replace the product free of charge or refund the purchase price.
BIOREBA AG shall not be liable for any direct or indirect consequential damage of any kind resulting from the use of our products.

2. Intended use

The PCR macroarray potato virus kits are validated for potato leaf and tuber samples (peel, stolon and sprouts). Dormant tubers can be pooled for rapid extractions.

The kits are available in two different sizes: kit 820032 for 96 tests and kit 820026 for 192 tests. Both can be used for single samples and for pooled samples of up to 10 tubers as shown in table 1.

3. Format, content, storage condition and quality

Art. No.	Description	Volume / Amount	Storage
820032	**PCR macroarray potato virus kit 96 / 10x includes:**		
610001	Rapid extraction buffer EB 1 potato (p) 100 ml	100 ml	RT
610007	Rapid extraction buffer EB 2 potato (p) 50 ml	50 ml	RT
450100	Extraction bags Standard 12x15 cm	100 pcs	RT
730021	Mix for PCR macroarray (pv) 1.2 ml	1.2 ml	-20 °C
630013	RT for PCR macroarray 14 µl	14 µl	-20 °C
630008	Taq for PCR macroarray 40 µl	40 µl	-20 °C
730027	Strep-AP for PCR macroarray 12.5 µl	12.5 µl	-20 °C
620024	HA buffer for PCR macroarray 12 ml	12 ml	+4 °C
620025	B buffer for PCR macroarray 12 ml	12 ml	+4 °C
620026	NBT/BCIP for PCR macroarray 0.1 ml	0.1 ml	+4 °C
620007	C buffer for PCR macroarray 80 ml	80 ml	+4 °C
610004	Wash buffer for PCR macroarray 200 ml	200 ml	RT
720023	Strips for PCR macroarray (pv) 12 pcs	12 pcs	+4 °C
410004	Adhesive film for PCR macroarray	2 pcs	RT
820026	**PCR macroarray potato virus kit 192 / 10x includes:**		
610006	Rapid extraction buffer EB 1 potato (p) 200 ml	200 ml	RT
610002	Rapid extraction buffer EB 2 potato (p) 100 ml	100 ml	RT
450100	Extraction bags Standard 12x15 cm	2 x 100 pcs	RT
730021	Mix for PCR macroarray (pv) 1.2 ml	2 x 1.2 ml	-20 °C
630012	RT for PCR macroarray 28 µl	28 µl	-20 °C
630008	Taq for PCR macroarray 40 µl	2 x 40 µl	-20 °C
730002	Strep-AP for PCR macroarray 25 µl	25 µl	-20 °C
620020	HA buffer for PCR macroarray 24 ml	24 ml	+4 °C
620006	B buffer for PCR macroarray 25 ml	25 ml	+4 °C
620023	NBT/BCIP for PCR macroarray 0.2 ml	0.2 ml	+4 °C
620007	C buffer for PCR macroarray 80 ml	80 ml	+4 °C
610004	Wash buffer for PCR macroarray 200 ml	200 ml	RT
720023	Strips for PCR macroarray (pv) 12 pcs	2 x 12 pcs	+4 °C
410004	Adhesive film for PCR macroarray	4 pcs	RT

Table 1: Format, content and storage conditions

Quality

Lot to lot consistency of the PCR macroarray potato virus kit is performed against predetermined specifications to ensure consistent product quality. See lot dependent certificate of analysis provided with the shipment.

4. Materials and equipment (not provided)

Equipment

- Microcentrifuge (capable of ≥ 6000 x g)
- Tissue homogenizer (e.g. homogenizer hand model Art. No. 400010 or HOMEX 6 Art. No. 400005)
- Micro pipettes
- Thermocycler
- Thermo shaker for hybridization (Art. No. 910030)
- Vortex
- Washer (optional)
- Reader (optional)
- -20 °C freezer and ice box

Consumables

- RNase-free filter tips
- RNase-free polypropylene centrifuge tubes with caps, capable of withstanding centrifugal forces of 6000 x g
- RNase free PCR tubes
- Disposable latex or vinyl gloves

5. Protocol

Please pay attention to the following points:

- The protocol in this manual must be followed
- Create an RNase free environment by cleaning the bench with 1 % bleach followed by 70 % ethanol
- Gloves must be worn at all times
- Use nuclease free tubes and filter tips
- Wear appropriate protective eyewear and clothing

5.1 DNA / RNA extraction

Put 0.2 g potato tissue into a clean grinding bag

Gently shake the rapid EB 1* and for single tests pipette** 500 µl rapid EB 1 into the grinding bag

Homogenize the potato tissue with a grinder

Transfer 100 µl homogenate avoiding the debris into a clean tube

Incubate the tube at 99.9 °C for 2 min then at 85 °C for 13 min and place immediately on ice

Spin the samples for 30 sec at 6000 g

Transfer 10 µl of supernatant without disturbing the pellet into a fresh tube containing 190 µl rapid EB 2

Vortex the sample briefly

Proceed with subsequent methods***

*Before shaking the buffer can show two phases
**For pools of 10 tubers use 1 ml EB 1
***For optimal results proceed with subsequent methods immediately

5.2 One-step Reverse Transcription PCR (RT-PCR)

Prepare a master-mix for a total of n+1 reactions (n is the number of samples, see Table 2), mix it well and keep on ice.

	Mix (pv) (Green lid)	Taq (Red lid)	RT (Blue lid)	Total volume
Volume (µl)	(n+1)*10	(n+1)*0.3	(n+1)*0.1	(n+1)*10.4

Table 2: Preparation of master-mix

For each PCR reaction transfer 10 µl master-mix and 2 µl of RNA sample into a PCR tube and cycle the samples in a thermocycler according to the program below (Table 3).

Step	Temperature	Time
Reverse Transcription	45 °C	30 min
Initial Denaturation	94 °C	2 min
30 Cycles	94 °C	15 sec
	58 °C	40 sec
	72 °C	40 sec
Final Elongation	72 °C	7 min
Hold	8 °C	

Table 3: PCR cycling conditions

5.3 Hybridization labeling and development

Bring the HA buffer, B buffer and C buffer to room temperature and mix them well. Keep Strep-AP and NBT/BCIP always on ice.

Pre-heat the thermo shaker to 55 °C

Heat the samples after the RT-PCR at 95 °C for 3 min 30 sec in the thermocycler and place on ice

Transfer 100 µl HA buffer into each well of the macroarray plate and add 10 µl of RT-PCR sample. When loading of the plate is completed, seal with adhesive film

Put the plate on the thermo shaker for 40 min at 55 °C and 1000 rpm

Carefully remove the film and discard the liquid. Wash the wells 3 times with 200 µl Wash buffer. When washing the plate for the third time, leave the liquid in the plate for 1 min

Discard the third wash and tap the plate upside down on paper towels repeatedly to remove residual liquid

Prepare the blocking reagent with Strep-AP and B buffer in the ratio 1:1000 for a total of n+1 reactions (n is the number of samples) (Table 4)

Add 100 µl blocking reagent into each well and incubate at 55 °C without shaking for 20 min

Discard the blocking reagent, add 200 µl Wash buffer per well and wait for 1 min. Repeat with 200 µl C buffer

Discard the content of the wells and tap the plate upside-down on a paper towel repeatedly to remove residual liquid

Prepare the detection reagent with NBT/BCIP solution and C Buffer in the ratio 1:100 for a total of n+1 reactions (n is the number of samples) (see Table 5)

Add 100 µl detection reagent into each well and incubate at room temperature in the dark for 7 min

Discard the liquid, wash the plate with tap water.
Air dry the plate and evaluate the results

Blocking reagent
Prepare blocking reagent for a total of n+1 reactions
(n is the number of samples).

Reagent	Volume (µl) / reaction	Volume (µl) / (n+1) reactions
B Buffer	100	100*(n+1)
Strep-AP	0.1	0.1*(n+1)
Blocking reagent	100.1	100.1*(n+1)

Table 4: Preparation of blocking reagent

Detection reagent
Prepare detection reagent for a total of n+1 reactions
(n is the number of samples).

Reagent	Volume (µl) / reaction	Volume (µl) / (n+1) reactions
C Buffer	100	100*(n+1)
NBT/BCIP	1	(n+1)
Detection reagent	101	101*(n+1)

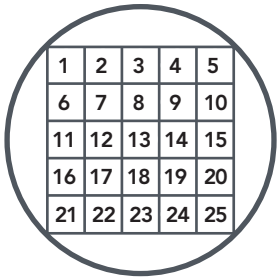
Table 5: Preparation of detection reagent

6. Evaluation

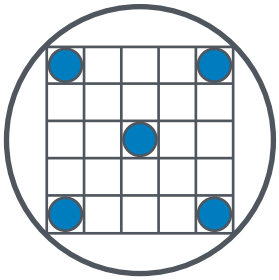
The kit encloses a plate (kit 192 has 2 plates) with 12 strips of 8 wells. Each well contains a macroarray to detect eight potato pathogens simultaneously. The 25 hybridization positions are arranged in a square of 5 by 5 (Figure 2 a).
On the array, the hybridization positive controls are in the four corner positions 1, 5, 21 and 25 while the RT-PCR control is in the center, position 13 (Figure 2 b).
A set of samples from healthy, virus-free plant tissue will show only the four hybridization positive control spots and one RT-PCR positive control spot (Figure 2 b).

Samples from infected potatoes will have visible spots in the corresponding positions on the array (Figure 2 e-m).
If there is no RT-PCR positive control spot (host gene control) (Figure 2 c), the test is invalid and requires repetition.
If there is no RT-PCR positive control spot together with the control hybridization spots seen but one or several test sample spots, the test is valid (Figure 2 d).

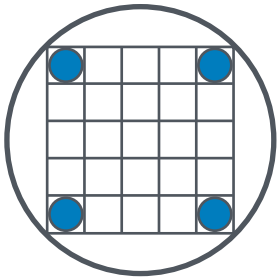
Arrangement, healthy plant and invalid / valid data



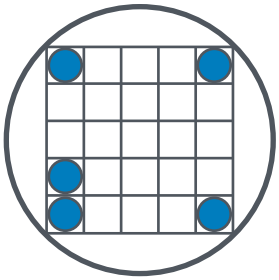
2 a) Arrangement



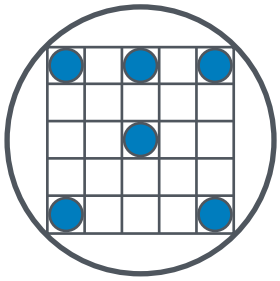
2 b) Healthy



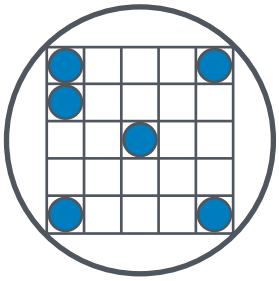
2 c) Invalid data



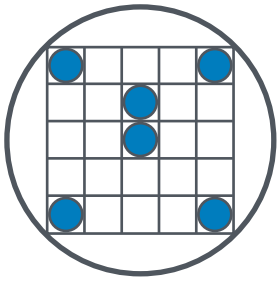
2 d) Valid data



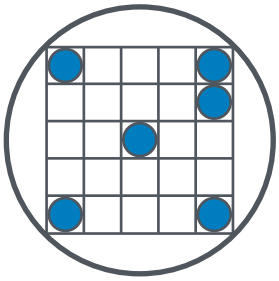
2 e) PVA



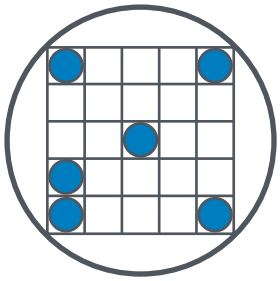
2 f) PVM



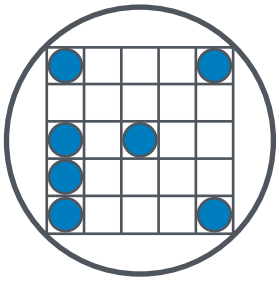
2 g) PVS



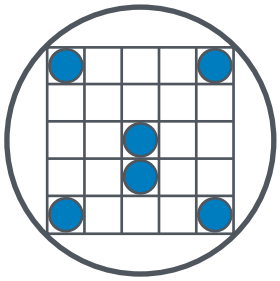
2 h) PVX



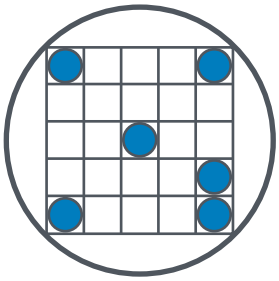
2 i) PVY



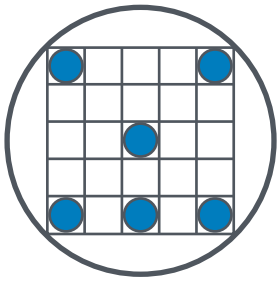
2 j) PVY-N Type



2 k) PLRV



2 l) PMTV



2 m) PSTVd

Figure 2: Scheme of analysis

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