BF-PFBV-ELISA

Enzyme Linked Immuno Sorbent Assay

for the Detection of

Pelargonium Flower Break Virus (PFBV)

Art.-No. KPE001

1. Introduction
The BF-PFBV-ELISA is a sandwich enzyme immunoassay which enables the detection of a PFBV infection of pelargoniums (geraniums). It is easy to perform and provides the result within one hour.

The detection limit of the test was established by extracting infected plant tissue (Chenopodium quinoa) and measuring dilutions of the extract. The virus could still be detected in dilutions of up to 1:1000 of a 10% extract.

The test allows analysis of 14 samples. It contains a positive and a negative control, immobilised on the solid phase on which the immunological reactions take place. These controls check the proper performance of the assay.

2. Precautions, disposal
Sample buffer and conjugate contain Bronidox as a stabiliser. The substrate contains tetramethylbenzidine and hydrogen peroxide.

All reagents are harmful only to such a low degree that no risk- and safety-phrases apply. Nevertheless, the reagents should not be swallowed and should be kept away from children. Avoid skin contact and rinse accidentally wetted spots with water.

After use, the reagents can be disposed of as waste water and poured into a sink. All solid components of the test are disposed of as ordinary garbage.

3. Contents of the kit
* Solid phase: This is a plastic strip with 16 wells, arranged in 2 columns. The solid phase together with a desiccant is hermetically packed in an airtight foil laminate pouch.
* 14 extraction bags for homogenisation of the samples
* 14 folded filters for clarifying the extracts
* Vial with 50 mL sample buffer (yellow, ready to use)
* 14 pipettes for the samples, 1 labelled pipette for each if the sample buffer, conjugate and substrate. The pipettes are graduated.
* Vial with 2,5 mL conjugate (red, ready to use)
* Vial with 2,5 mL substrate (colourless, ready to use). The vial is black in order to protect the substrate from light.

The test is stable at least until the expiry date stated on the packaging. It has to be stored at about 4°C.

4. Accessories required but not supplied
* 1 glazed pestle to homogenise the samples, 14 funnels, 14 reagent tubes and 1 tube rack (Set: Art.-No. Z001). All components are intended for repeated use.
* A pair of scissors, cold tap water, adsorbent paper

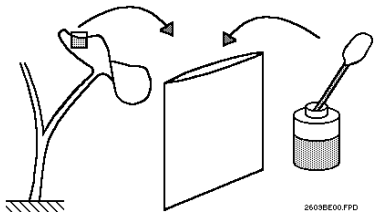
5. Principle of the test
The 2 x 8 wells of the solid phase are coated with a polyclonal antibody which specifically recognises PFBV. In addition, a negative (PFBV-free) and a positive (PFBV-containing) sample are immobilised in 2 wells on the edge. These are the control wells; they are marked green and red, respectively.

First reaction: The wells (except the control wells) are loaded with the samples, i.e. extracts from geranium plants. PFBV antigens eventually present in the sample are bound to the immobilised antibody, forming the antigen-antibody complex.

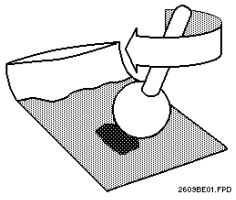
Second reaction: After a washing step which flushes all non-bound sample components from the solid phase, a second PFBV-antibody is added; the control wells are included. This antibody is conjugated with peroxidase ("enzyme conjugate") and binds to the immobilised antigen-antibody complex.

Third reaction: After a further washing step which removes non-bound conjugate from the solid phase, colourless substrate is added; the control wells are included. It is converted into a blue product by the conjugate. PFBV-infected samples exhibit the blue colouration (like the positive control), whereas non-infected samples remain colourless (like the negative control).

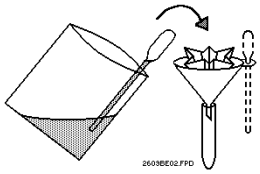
6. Preparation
Samples: Cut out 14 pieces of geranium leaves (about 10 cm² each) and put them into the vertically placed extraction bags. Note the designation of the samples on the corresponding bag and numerate according to the solid phase (see table p. 6): B1-H1, B2-H2. Then, using the sample buffer pipette, add about 3 mL (corresponds to the uppermost pipette mark) sample buffer.



Place the extraction bag on an even surface, the opening facing upwards. Homogenise the leaf by rubbing the glazed pestle in circles over the bag, applying moderate pressure. When the extract turns green (release of chlorophyll), the extraction process is completed.

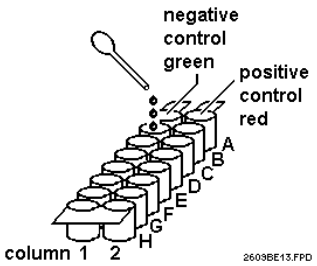


Transfer the extracts one by one, each with a separate sample pipette, into separate folded filters. Collect the filtrates into numbered reagent tubes. These filtrates are the actual test samples. The pipettes will be used again and must not be mixed up!



7. Assay procedure
Before starting the test, all reagents should have reached room temperature (19-29°C). All reactions are performed at room temperature.

Incubation with samples: Cut open the pouch with the solid phase and discard the desiccant bag. Insert the solid phase into its support (tray of the box), the green and red marked control wells facing upwards. Dispense the filtrates with the appropriate sample pipettes into the wells; 3 drops in each. Omit the control wells which remain empty during the initial reaction step! The incubation takes 10 minutes.

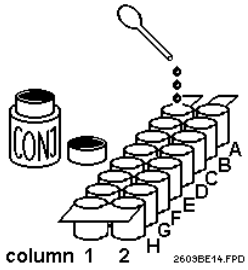


Note the identity of the samples:

	Column 1	Column 2
Line A	neg.-Ctrl. (green)	pos.-Ctrl. (red)
Line B	_____	_____
Line C	_____	_____
Line D	_____	_____
Line E	_____	_____
Line F	_____	_____
Line G	_____	_____
Line H	_____	_____

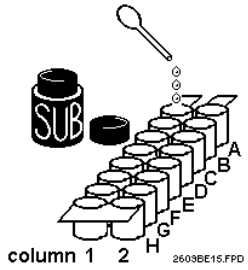
Washing the solid phase: Take the solid phase out of the support and pour its contents into a sink with a flinging movement. Then, wash the entire solid phase **thoroughly** (3 times for about 5 seconds) under **cold** tap water. Finally, empty the solid phase, remove adherent water by shaking and tapping on adsorbent paper. Replace the solid phase into its support.

Incubation with conjugate: Dispense the red conjugate with the conjugate pipette into the wells, including the control wells; 3 drops per well. The incubation takes 10 minutes.

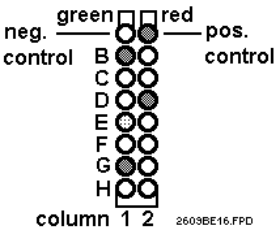


Washing the solid phase: as described above.

Incubation with substrate: Dispense the colourless substrate with the substrate pipette into the wells, including the control wells; 3 drops per well. The incubation takes 10 minutes. As the substrate is photosensitive, avoid intense light exposure (e.g. direct sunlight) during incubation.



8. Evaluation
The assay has been conducted properly if at the end of the substrate incubation the positive control exhibits an intense blue colour, whilst the negative control remains colourless. The result could look something like this:



In this case, samples 1B, 1G and 2D are strongly infected by PFBV, sample 1E is weakly infected and all other samples probably originate from healthy plants.

9. Warranty and liability
BF-BIOlabs guarantees that the delivered product has been thoroughly tested in order to ensure that the specifications are fulfilled and that it conforms to the properties specified here. No further warranties are given. In particular, no liability can be accepted for any damage which results from inappropriate storage or use of this product.

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