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Protocol for the use of lyophilized positive control (VPC) of plant pathogenic virus

Description of the control

- A control consists of 1 vial containing lyophilized plant material and partially purified virus.
- o The colour of the contents of the vial will be green, brown, yellow or white.
- The consistency of the contents of the vial will be powdery, sticky or lumpy¹.
- o Upon arrival the vials should be stored in the refrigerator (4-10°C) until use.
- Use the lyophilized control within 1 year.

Preparation and using the control

- Prepare the working solution of the control after the sample preparation but before washing the ELISA plate.
- Add 1.2 ml of demineralized water to 1 vial of lyophilized control.
- $\circ\quad$ Leave the vial at room temperature for circa 5 minutes.
- Mix the contents of the vial by hand and shaking gently².
- o The control is now ready for use.
- We call this the working solution of the positive control.
- Keep the working solution of the positive control cool, on ice or in a refrigerator, until use.
- The working solution of the positive control can be used without further diluting.
- o The working solution of the control should be used within 1 day.
- The working solution of the control can be used in DAS-ELISA, Immuno fluorescence (IF and IIF) and Luminex xMAP assays.
- Add the working solution of the positive control to the assay at the same time and in the same amount as the samples.

Additional storage information about the lyophilized control

- o The **lyophilized** positive control should be stored at in the refrigerator.
- The **lyophilized** positive control has a shelf-life of 1 year after date of purchase.

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¹ The colour and consistency of the lyophilized control is dependent on the plant material used as a production host.

² Lyophilized controls are provided in a vial large enough to contain the prepared liquid.

Additional storage information about the working solution

- The working solution of the control should be used within 1 day after preparation.
- The working solution of the control should be kept cool on ice or in a refrigerator until use.
- It is possible to prepare smaller aliquots (e.g. 205 ul) from the working solution of the control and store these aliquots at -20°C during maximally 1 month.

Long-time storage of the working solution

If the **working solution** of the control needs to be stored for a longer period of time than 1 month it is recommended to prepare the lyophilized control in a different way:

- Add 540 ul of glycerol and 660 ul of demineralized water to the lyophilized control.
- Leave the vial at room temperature for circa 5 minutes.
- Mix the contents of the vial gently by hand and turning the vial upside down several times.
- o Divide the solution immediately after mixing into useful smaller aliquots³.
- o Store these aliquots of **working solution** of the control at -20°C until use.
- The aliquots of working solution of the control should be used within 1 year after date of purchase.
- The aliquots working solution of the control can be used in an assay without further dilution.

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³ Use reverse pipetting as a pipetting technique