

# Manual for sampling of plant material for virus tests



Many virus tests nowadays are performed using sensitive methods. This enables potential infections to be detected at an early stage. This manual provides information about hygiene, sampling for PCR and ELISA and the correct way to submit samples.

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## Hygiene

### Why is hygienic sampling so important?

Many virus tests nowadays are performed using sensitive methods. This enables potential infections to be detected at an early stage. Virus testing can also detect any cross-contamination between (sub)samples. This is why hygiene during sampling is very important. By using the proper method you can prevent cross-contamination during sampling.

### Why should sample material be submitted in Bioreba bags?

If possible, always submit samples of plant material in Bioreba extraction bags measuring 12 x 15 cm. You can order these through the relevant suppliers. For small quantities (up to 100 bags) please contact Naktuinbouw, tel. +31 (0) 71 332 6262.

Samples are tested in Bioreba bags in the laboratory. The advantage of submitting your samples in Bioreba bags is that it reduces the risk of cross-contamination and allows a faster processing time at the laboratory.

When submitting a sample, make sure the material is suitable for testing. This means that the leaves should not be brown, dehydrated, mouldy or necrotic. Send material that is showing symptoms to Naktuinbouw, Diagnostics Team.

## Method

- Prepare the required materials:
  - Bioreba extraction bag(s) and outer bag or box.
  - Disposable gloves or plastic bags.
  - A fully completed submission form.
- Wear a new pair of disposable gloves for each (sub)sample (each Bioreba bag) or fold a clean plastic bag around the hand you use to take the sample.
- Take the (sub)sample.
- Collect the material in the Bioreba bag at the front of the filter (this is the side with the text box).
- Number the Bioreba bags using the text box or place a sticker in the centre of the front of the bag. Do not use this sticker to seal the bag.
- **Important:** the number of leaf samples must not exceed the specified quantity, more sample material will interfere with the analysis. Only sample the prescribed quantity.

## Submitting samples

Please include a fully completed submission form with your samples. You can find this form on the Naktuinbouw website under: [Submission requirements](#).

Submitting samples correctly: Bundle the Bioreba bags in numerical order using elastic bands. Leave the bags open and pack them in a sealed box.

### Post address for samples:

Naktuinbouw  
Attn. Laboratories Administration  
P.O. Box 40  
2370 AA Roelofarendsveen

### Visitors address:

Naktuinbouw, Sotaweg 22  
2371 GD Roelofarendsveen



## Sampling for PCR

The number of leaves varies according to the crop variety for sampling for PCR analysis. The table below indicates the number of leaves you can submit per bag for PCR analysis.

| Crop                    | Sample material                                       | Number of leaves per bag | Maximum total weight per bag | Specifics                      |
|-------------------------|-------------------------------------------------------|--------------------------|------------------------------|--------------------------------|
| Ornamental crops        | Leaf (pieces)                                         | 1 – 25                   | 1 gram                       |                                |
| Phalaenopsis            | Leaf or pieces of stem                                | 1 – 25                   | 1 gram                       |                                |
| Tissue culture material | Leaf from material that has grown as much as possible | 1 – 25                   | 1 gram                       | Material must be free of agar. |
| Freesia 5 x 10          | Leaf tips                                             | 1 – 10                   | 1 gram                       |                                |
| Freesia 3 x 50          | Leaf tips                                             | 1 – 50                   | 1 gram                       |                                |

Examples of samples of ornamentals submitted correctly for PCR analysis:



Petunia 0.3 gram



Helleborus 0.3 gram



Hebe 0.9 gram



Lavendel 0.2 gram



Phalaenopsis 0.3 gram



Bacopa Sutura 1 gram



Dahlia 1 gram



Petunia 1 gram



Chrysant 1 gram



Tissue culture samples



Tissue culture samples



Tissue culture samples

## Sampling for ELISA

The number of leaves varies according to the crop variety for sampling for ELISA analysis. The table below indicates the number of leaves you can submit per bag for ELISA analysis.

| Crops                   | Sample material                                                          | Number of leaves per bag | Maximum total weight per bag | Specifics when submitting tissue culture material                                                                                                                                                                                                                                                       |
|-------------------------|--------------------------------------------------------------------------|--------------------------|------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ornamental crops        | Leaf (pieces)                                                            | 1 – 2                    | 0.5 gram                     | Preferably use in vivo (propagating) material or in vitro material that has grown. Do not use material that has just been transferred.<br><br>With tissue culture material, clones are tested individually. The leaf or the entire plant without the root is used.<br><br>Material must be free of agar |
| Phalaenopsis            | Pieces of leaf or stem                                                   | 1 – 2                    | 0.5 gram                     |                                                                                                                                                                                                                                                                                                         |
| Tissue culture material | Leaf from material that has grown as much as possible. One clone per bag | 1                        | 0.5 gram                     |                                                                                                                                                                                                                                                                                                         |
| Alstroemeria            | Leaves                                                                   | 1 – 3                    | 0.5 gram                     |                                                                                                                                                                                                                                                                                                         |
| Pelargonium             | Only stems, no leaves                                                    | 1 – 2                    | 0.5 gram                     |                                                                                                                                                                                                                                                                                                         |

**Important:** Are you submitting material for potyvirus testing as well as ELISA? Please submit the samples in two separate Bioreba bags: One bag for the 'standard' ELISA and one bag containing two leaves for the potyvirus test.

Examples of samples of ornamentals submitted correctly for ELISA analysis:



Alstroemeria



Callibrachoa



Campanula



Helleborus



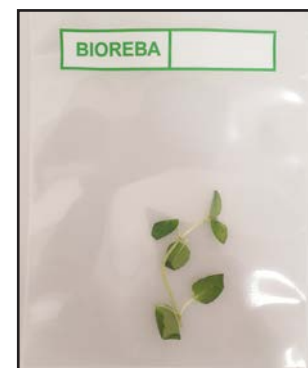
Pelargonium (leaf stem)



Phalaenopsis (flower stem)



Tissue culture samples



Tissue culture samples