Exymes Quick-Start Guide

DNA Extraction Using

*phyto*GEM

Ultra Fast DNA Extraction

Find more information at
www.exymesplc.com

or email
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QSG012

*phyto*GEM™

*phyto*GEM is for DNA extractions from sample types primarily from plants including leaves, stems, roots and pathogens. More information can be obtained from www.exymesplc.com

General instructions

- All manipulations should be performed in a clean-room or a PCR hood.
- Labcoats, gloves and hairnets should be worn at all times.
- Use only certified DNA-free tubes and reagents.
- Wash any equipment that will come into contact with the sample in 0.05% hypochlorite bleach. Rinse thoroughly with DNA-free water.

Reagent storage: *phyto*GEM reagents are stable at room temperature but on arrival should be stored at 4°C. After tubes have been opened, the *phyto*GEM should be placed at -20°C. This is to safeguard against accidental contamination. The buffer can remain at 4°C for convenience.

Histosolv: is a mixture of reagents that weaken tissue cell walls. It is delivered as a lyophilised powder. This should be resuspended in DNA-free water as follows:

Kit size (rxn)	Code	Volume of water to add
100	PHY0100	1.1 ml
500	PHY0500	5.5 ml
1000	PHY1000	11.0 ml

Procedure overview: Exymes extraction products use a unique mixture of thermophilic and mesophilic enzymes. Where a low temperature stage is used, mesophilic cell wall degrading enzymes come into play. The 75° step then activates a thermophilic proteinase that lyses the cells, kills nucleases and strips the DNA of nucleosomes. A final 95° step deactivates the thermophilic proteinase.

Extraction of DNA from storage cards

Sample Types:

- Plant leaves, stems and roots
- Plant Pathogens

READ THE TECHNICAL TIPS AT THE END OF THIS DOCUMENT

1. Completely thaw *phyto*GEM and Histosolv and mix by gently inverting the tubes. Remove GREEN+ buffer and the Enhancer from the refrigerator and mix.
2. Prepare the following 100 µl extraction[1] mixture:
    - 10 µl GREEN+ buffer
    - 1 µl *phyto*GEM
    - 10 µl Histosolv
    - 10 µl Enhancer
    - 69 µl DNA-free water
3. Dispense 100 µl of master mix into each 0.2 ml tube.
4. Take a sample by crushing the plant onto the storage cards using the crusher tool.

IF USING MULTI-USE PUNCHES, RINSE THE PUNCH IN DILUTE BLEACH AND THEN ETHANOL. BLOT DRY.

5. Take 1 - 2 punches[2] from your storage card and drop them in the 0.2 ml tube with the master mix. Make sure that the punches are completely submerged in the reagents.
6. Close the 0.2 ml PCR tube and place into a thermal cycler.
7. Run the following thermal program[3,4]:
    - 52°C 5 mins.
    - 75°C 5 mins.
    - 95°C 2 mins.
8. Remove samples from the thermal cycler. Aspirate the extract into a fresh 0.2 ml and discard the punches. Store at 4°C.

[1] Volumes can be altered through internal laboratory optimisation.

[2] Number of punches can be adjusted through internal laboratory optimisation.

[3] Times may be adjusted by internal laboratory optimisation.

[4] Changes to the default temperatures are not recommended.

Extraction of DNA from homogenised leaves, ground plant tissue

Sample Types:

- Homogenised plant leaves, stems and roots
- Plant Pathogens

READ THE TECHNICAL TIPS AT THE END OF THIS DOCUMENT

1. Weigh 5-10 mg of leaf and place in a 1.6 ml tube. Add 100 µl of ultrapure water and homogenise using a sterile hand-held homogenising tool, tissue lyser or a mortar and pestle.

Ensure the sample is homogenised completely. For ground bark, woody tissue or roots, make a suspension of 5 - 10 mg ground tissue in 100 µl ultrapure water. Prepare and manage on ice or at 4 °C for best results.

2. Prepare the following 100 µl extraction[1] mixture in a 0.2 ml PCR tube:
    - 10 µl 10x GREEN+ buffer
    - 1 µl *phyto*GEM
    - 10 µl Histosolv
    - 10 µl Enhancer
    - 64 µl Ultrapure water
    - 5 µl Leaf homogenate or tissue suspension[2]

Cut the ends off the pipette tips to help transfer the homogenate.

3. Mix thoroughly by vortex. Place the 0.2 ml tube into a thermal cycler.
4. Run the following thermal program[3,4]:
    - 52°C 5 mins.
    - 75°C 5 mins.
    - 95°C 2 mins
5. Remove the tube from the thermal cycler and briefly centrifuge the tube to collect the plant tissue to the bottom of the tube.
6. Aspirate the supernatant to a fresh 0.2 ml tube. Store at 4°C.

[1] Volumes can be altered through internal laboratory optimisation.

[2] Amount of homogenate can be adjusted through internal laboratory optimisation.

[3] Times may be adjusted by internal laboratory optimisation.

[4] Changes to the default temperatures are not recommended.

Extraction of DNA from fungal cultures

READ THE TECHNICAL TIPS AT THE END OF THIS DOCUMENT

1. Transfer a small amount of fungal culture into a 1.6 ml Eppendorf tube using an inoculation loop or tease tool (half a loopful of sample is usually sufficient for good DNA yield). Add 200 µl of ultrapure water and homogenise using a sterile hand-held homogenising tool or tissue lyser. Prepare and manage on ice or at 4 °C for best results.

2. Prepare the following 100 µl extraction[1] mixture in a 0.2 ml PCR tube:
    - 10 µl 10x GREEN+ buffer
    - 1 µl *phyto*GEM
    - 10 µl Histosolv
    - 10 µl Enhancer
    - 69 µl Fungal homogenate[2]
3. Mix thoroughly by vortex. Place the 0.2 ml tube into a thermal cycler.
4. Run the following thermal program[3,4]:
    - 52°C 5 mins.
    - 75°C 5 mins.
    - 95°C 2 mins
5. Remove the tube from the thermal cycler and briefly centrifuge the tube to collect the plant tissue to the bottom of the tube.
6. Aspirate the supernatant to a fresh 0.2 ml tube. Store at 4°C.

[1] Volumes can be altered through internal laboratory optimisation.

[2] Amount of homogenate can be adjusted through internal laboratory optimisation.

[3] Times may be adjusted by internal laboratory optimisation.

[4] Changes to the default temperatures are not recommended.

Technical Tips

- *phyto*GEM is a preparative method for DNA extraction. The method lyses cells and removes nucleoproteins from the DNA. Extracted DNA can be used for many types of genotyping including SNP and STR analysis as well as quantitative, multiplex and end-point PCR.

- There is no concentration step in the procedure and so the concentration of the extract is dependent on: 1) The quality of the sample; 2) In the case of swabs, the type of swab and the volume of water used to wash the swab; 3) The extraction volume (which in some cases can be scaled).

- DNA extracted using *phyto*GEM is largely single-stranded because of the 95°C heat step.

- For accurate yield assessment, a qPCR is recommended. If standard fluorescent chelating dyes are to be used for normalising samples, then we recommend taking a sample of the extract before the 95°C step (See Exymes Application Note 109). Alternatively, you can generate a standard curve using a previously-made extract that has been quantified.

- As with any preparative method for nucleic acid extraction, best results are obtained when samples are handled at 4°C, or on ice, before and after extraction.

- For long term storage of the extracted DNA, add TE buffer to 1x (10 mM Tris, pH 7.5, 1 mM EDTA) and store at -20°C. TE buffer is an optional add-on for the kit.

The *phyto*GEM reagents are stable at room temperature, but after tubes have been opened and for longer term storage, the enzymes should be stored at -20°C and the buffers at 4°C.

More information can be found at:
www.exymesplc.com

If you still need help, email us at:
info@exymesplc.com

*QSG012 phyto*GEM v1.0