Clear® Detections
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User Manual

Banana Plant Tissue DNA Extraction & Purification Kit

EX-P-P-PDEP

For general laboratory and research use only

Content

We recommended that you read the entire manual before starting the procedure.
Feel free to contact us at info@cleardetections.com for questions regarding this protocol, laboratory set-up or equipment specifications.

1. Introduction

This ClearDetections Plant DNA extraction & purification kit is designed to extract and purify DNA from banana plant tissue. The kit extracts and separates DNA from proteins, detergents and low molecular weight compounds. The purified DNA is suitable for diagnostic purposes, including *Fusarium oxysporum* f. sp. cubense TR4 detection using ClearDetections Real-Time PCR FocTR4 diagnostic kit.

2. Kit components, storage conditions, and shelf life

Components	Code	Storage
Extraction buffer	PEB	4 °C
Protease	PR	4 °C
Homogenization solution	HS	4 °C
Equilibration solution	ES	4 °C
RNase A	RNA	4 °C
DTT	DTT	4 °C (-20°C after first use)
SDS	SDS	15-30°C
DNA purification plate(s)	PWP	15-30°C
DNA collection plate(s)	DCP	15-30°C

During the shipment all kit components are stable at room temperature. After arrival, store the components accordingly. If properly stored, the kit can be used until the expiry date printed on the box.

3. Additional reagents and equipment needed

Extraction and purification:

- Vortex
- Centrifuge
- Temperature controlled incubator/heat block or water bath
- Bead beater and stainless steel beads (2 to 3 mm), or pestle and mortal
- Pipettes and corresponding filter-tips for volumes of 5-1000 µL

Solvents and reagents

- Optional: liquid nitrogen

4. Protocol for banana plant DNA extraction & purification

4.1 Sample preparation

Sampling the banana tissue is a crucial step that greatly influences the success of downstream processes like Foc TR4 detection by Real-Time PCR. For Foc TR4 diagnostic purposes, use 250 mg of vein tissue isolated manually from the inner leaf of the pseudostem or 250 mg of corm tissue (where the infection is most pronounced).

4.2 DNA extraction

Note: Before starting this procedure, make sure you have enough time to conclude DNA extraction & DNA purification in one go. After the extraction is complete, the DNA needs to be purified immediately to avoid possible degradation. After purification, the DNA can be safely stored at - 20°C.

1. Pre-heat the incubator or water bath to 60 °C.

2. Prepare **homogenization mix** sufficient for all samples:

The homogenization mix:	per sample (µL)	x 10 samples*
Homogenization solution	185	2035
DTT	3	33
RNase	12	132

* When calculating the volume of the homogenization mix, we advise increasing the number of samples by approximately 10% to account for pipette error.

3. To proceed with DNA extraction using bead beater go to section A. To proceed with DNA extraction using pestle & mortar go to section B.

A. Grinding with bead better & stainless-steel beads:

a. Transfer up to 250 mg banana plant tissue into tube and add 1 or 2 beads.

Note: to increase DNA extraction efficiency, the grinding of the plant tissue can be done in liquid nitrogen. For this, chill the tubes containing plant material in liquid nitrogen before bead beating. Be sure the lid is closed properly, to not let liquid nitrogen enter in the tube.

b. Grind 1 minute at 30Hz in the bead beater. Afterwards, spin down briefly (5 seconds) to make sure all material is at the bottom of the tube, so the lid can be opened without losing the sample.

c. Add 200 µL of freshly prepared **homogenization mix** and grind again 1 minute at 30Hz in the bead beater. Afterwards, spin down briefly (5 seconds).

Note: Do not freeze samples again in liquid nitrogen at this step. The sample including buffer should not be frozen, as this would block efficient bead beating.

d. Proceed to point 4 of DNA extraction procedure.

B. Grinding using a mortar & pestle:

a. Transfer up to 250 mg banana plant tissue into a mortar.

Note: to increase DNA extraction efficiency, the grinding of the plant tissue can be done in liquid nitrogen. For this, chill the mortar with liquid nitrogen before grinding. After adding the banana plant tissue, add enough liquid nitrogen to freeze your sample, and grind plant tissue vigorously using a pestle.

b. Add 200 µL of freshly prepared **homogenization mix** and grind plant tissue vigorously using a pestle.

Note: Do not add liquid nitrogen after adding the buffer.

c. Transfer the content of the mortar to a 1.5 ml Eppendorf tube, compatible with the heat block.

Note: Be careful not to transfer any liquid nitrogen to the 1.5 ml Eppendorf tube when transferring the sample. If this is the case, let the liquid nitrogen evaporate before closing the tube.

4. Prepare **extraction mix** sufficient for all samples:

The extraction mix:	per sample (µL)	x 10 samples*
DNA extraction buffer	70	770
Protease	20	220
SDS	10	110

* When calculating the volume of the extraction mix, we advise increasing the number of samples by approximately 10% to account for pipette error.

5. Add 100 µL of freshly prepared **extraction mix** to each sample and vortex samples thoroughly.

6. Incubate the tubes for 1 hour at 60 °C and briefly vortex tubes every 10-15 minutes.

Note: Continue with equilibration of the DNA purification plate (step 1-4 of section 4.3: the DNA purification protocol) during this 1 hour incubation step.

7. Vortex tubes thoroughly and centrifuge for 1 min at 10.000 x *g*. After this step, samples are ready to be loaded on the equilibrated purification plate (step 5 of section 4.3: the DNA purification protocol).

Note: To facilitate easy pipetting of the supernatant, samples should not stand long after centrifugation. It is advisable to work with batches of 8 samples, so immediately after spinning down the supernatant can be loaded onto the equilibrated plate.

4.3 DNA Purification

1. Take the DNA purification plate with attached waste collection plate.

2. Add 350 µL **equilibration solution** to each well for every crude DNA extract to be purified.

3. Stand at room temperature for 5 min and then centrifuge for 1 min at 350-500 x *g*. Be careful to properly balance the centrifuge.

4. Discard the waste collection plate and **place the DNA purification plate to the DNA collection plate.**

5. Transfer 100 µL of each DNA extract (step 7 of section 4.2: the DNA extraction protocol) to the equilibrated DNA purification plate.

Note: Be careful, try not to disturb the pellet. See note at step 7 of section 4.2: the DNA extraction protocol.

6. Stand the DNA purification plate for 3 min at room temperature.

7. Centrifuge 1 min at 700 x *g*.

8. The flow-through captured by the DNA collection plate contains purified DNA ready for Real-Time PCR or storage.

Note: Dilute the DNA before proceeding to perform Real-Time PCR (see protocol RT-F-D-0901/0902).
Store the DNA between 4-8 °C for no more than several days. For long-term storage keep at -20 °C or less.

For further questions or assistance, please do not hesitate to contact us at info@cleardetections.com.

5. Notices and Disclaimers

Despite the utmost care in the development and preparation of this protocol, ClearDetections cannot take any responsibility for errors, omissions and/or future changes herein.

This kit is for general laboratory and research use only.

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