6. Take the tube/plates from the incubator or water bath and increase the temperature to 95°C.
7. Incubate the tube/plates 5 minutes at 95°C, to denature Proteinase K.
8. Vortex samples thoroughly and briefly centrifuge.
9. The samples are now ready for DNA purification.

DNA Purification

1. Preparation of the purification column/plate:
    a. Take the DNA purification column/plate together with attached waste collection tube/plate.
    b. Add 350 µL equilibration solution to each column/well and stand at room temperature for 5 min.
    c. Centrifuge for 1 min at 350 x g.
    d. Discard the waste collection tube/plate and place the DNA purification column/plate in the DNA collection tube/plate.
2. Pre-purification of the sample
    a. Take the DNA pre-purification tube/plate containing a white pellet. Transfer 200 µL of DNA extract to each tube/plate.
    b. Carefully re-suspend the white pellet by pipetting up and down until it has fully mixed with the sample.
    c. Centrifuge 1 min at 1.500 x g.
3. Transfer 100 µL of each sample from the DNA pre-purification tube/plate to the equilibrated DNA purification column/plate. Be careful not to disturb the pellet in the columns!
4. Stand the DNA purification tube/plate for 3 min at room temperature.
5. Centrifuge 1 min at 700 x g.
6. The flow-through contains purified nematode DNA ready for Real-Time PCR or storage. Storage is recommended at -20 °C.

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 designed for general laboratory and research use only. For the legal notices & disclaimer see website, www.cleardetections.com, or contact ClearDetections at info@cleardetections.com.

ClearDetections
revolutionary diagnostics

Nematode DNA extraction kit & purification kit for nematode suspensions and multiple cysts

EX-N-T/P-NDEP

For general laboratory and research use only

Introduction

This ClearDetections Nematode DNA extraction & purification kit is designed to extract genomic DNA from nematode suspensions and multiple cysts. The obtained DNA extracts can be used directly for downstream applications like Real-Time PCR using one of the ClearDetections Real-Time PCR nematode identification kits (RT-N-D/W-XXXX), see www.cleardetections.com.

The ClearDetections Nematode DNA extraction & purification kit is in tube/plate and plate format. This manual is applicable for both formats, but will have notes specifically for one of the formats.

Note: To extract DNA from individual nematodes and/or single cysts, we recommend the 'ClearDetections kit Nematode DNA extraction from individual nematodes and/or single cysts (EX-N-B-SNDE).

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.

Kit components, storage conditions, and shelf life

Components	Code	Storage conditions
Extraction buffer 2x	EXB_SUS	4°C
Proteinase K solution	PK_SUS	4°C
Equilibration solution	EQB	4°C
DNA pre-purification tubes/plates	PPT/PPP	Room temp.
DNA purification columns/plates	PWC/PWP	Room temp.
DNA collection tubes/plates	DCT/DCP	Room temp.

Reagents and equipment to be supplied by the user

- Vortex
- Fume hood
- Centrifuge for tubes/plates
- Temperature controlled incubator/heat block or water bath
- Pipettes and corresponding low adhesion* (e.g. siliconized) filter-tips for volumes of 5-1000 µL
- 50 mL centrifuge tube (for extraction of DNA from nematode suspensions)
- Low adhesion 2,0 mL microcentrifuge tube
- Pestle (for crushing cysts)
- Nuclease free water
- β-Mercaptoetanol (2-Mercaptoetanol) or Dithiothreitol 5.0 M (DTT) **

* Use of other tips may negatively affect the nematode detection.

** 5.0 M DTT can be either freshly prepared, or prepared in advance, stored at -20˚C, and used directly after defrosting.

Sample preparation

This ClearDetections kit is capable of processing large numbers of nematodes per sample; suspensions containing 10,000 nematodes, or up to 20 cysts. This protocol assumes that nematode suspensions or cysts have already been isolated from sample materials, with unwanted debris such as soil aggregates or plant tissues removed. For isolation of nematodes and/or cysts, please go to EPPO bulletin for nematode extraction (PM 7/119 (1) 2013, Nematode extraction. EPPO Bull, 43(3), 471-495).

Nematode suspensions:

1. Transfer the obtained nematode suspensions to 50 mL centrifuge tube.
2. Centrifuge for 10 min at 500 x g.
3. Carefully remove the supernatant with a pipette without disturbing the nematode pellet until approximately 0.5 - 2 mL liquid remains.
4. Re-suspend the nematode pellet in the remaining water, transfer it to a low adhesion 2 mL tube and then centrifuge for 5 min at 500 x g.
5. Carefully remove the supernatant with a pipette without disturbing the nematode pellet until approximately 150 µL remains.
6. The sample is now ready for DNA extraction and purification.

Multiple cysts:

1. Transfer up to 20 cysts into a clean 1.5 mL low adhesion microcentrifuge tube containing 150 µL deionized water.
2. Crush the cysts intensely using a pestle (disrupting the egg walls to free the larvae).

Note: It is possible to create a pestle from a low adhesion filter tip by sealing the tip using a flame. A ClearDetections video "How to crush nematode cysts" can be found at www.youtube.com demonstrating how to efficiently crush cysts using pipette tips as pestles.

3. The sample is now ready for DNA extraction and purification.

DNA extraction

1. Pre-heat the incubator or water bath to 65 °C.
2. Prepare an extraction mix sufficient for all the samples:

Extraction mix	Per sample (µl)	x 10 samples*
Extraction buffer 2x **	150	1650
Proteinase K	6	66
2-Mercaptoethanol /DTT	1.5	16.5

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

** If the extraction buffer has precipitated, re-dissolve at 65°C and cool down to room temperature before use.

3. Add 150 µL of extraction mix to each sample.

Note: Aim the pipette tip at the walls of the tube and add the buffer without touching the sample. Nematodes are statically charged and can stick to pipette tips!.

4. Close the tube and thoroughly re-suspend the pellet using a vortex.
5. Incubate the tube for between 2 – 3 hours at 65 °C. Briefly vortex the tube +/- every 30 min.

Note: This step is required to lyse nematodes and release their DNA.

Note: It is possible to already execute the preparation of the purification column/plate (4.3-1) just before the end of this incubation step.