Clear® Detections
revolutionary diagnostics

User Manual

Nematode DNA extraction & purification kit

Scope: Nematode suspensions and multiple cysts

For general laboratory and research use only

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 Nematode DNA extraction & purification kit is designed to extract and purify DNA from nematode suspensions and/or multiple cysts. The kit separates DNA from proteins, detergents and low molecular weight compounds. The purified nematode DNA is suitable for downstream applications such as PCR and Real-Time PCR.

Note: For DNA extraction from *individual* nematodes or cysts, we recommend using the "ClearDetections DNA extraction kit for individual nematodes and single cysts".

2. Kit components, storage conditions and shelf life

Extraction & purification	Storage
Extraction buffer	4 °C
Proteinase K solution	4 °C
Equilibration solution	4 °C
DNA pre-purification plate	Room temp
DNA purification plate	Room temp
DNA collection plate	Room temp

If properly stored, the kit components can be used until the expiry date printed on the box.

3. Additional reagents and equipment needed

Extraction and purification:

- Vortex
- Fume hood
- Centrifuge for plates/tubes
- Temperature controlled incubator or water bath
- Pipets and corresponding low adhesion* (e.g. siliconized) filter-tips for volumes of 10-1000 µL

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

For extraction of DNA from nematode suspensions:

- 50 mL centrifuge tubes
- Low adhesion 2.0 mL microcentrifuge tubes

For extraction of DNA from multiple cysts:

- Low adhesion 1.5 mL microcentrifuge tubes
- Pestle (for crushing cysts)

Solvents and reagents

- Nuclease free water
- β-mercaptoethanol (2-Mercaptoethanol) or freshly prepared 5.0 M Dithiothreitol (DTT)*
  *5.0 M DTT can be prepared in advance, stored at -20 °C and used directly after defrosting.

Feel free to contact us for advice and support if this situation applies to you.

4. Protocol for nematode DNA extraction & purification

4.1 Sample preparation

Our kit is capable of processing large numbers of nematodes per sample; suspensions containing 10,000 nematodes, or up to 20 cysts. Our 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. An example protocol for nematode isolation can be found in the video "Oostenbrink instructions (in English)" published by NEMA EDU on www.youtube.com.

DNA isolation from nematode suspensions:

1. Transfer the obtained nematode suspensions to 50 mL centrifuge tubes.
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. Sample is ready for DNA extraction and purification.

DNA isolation from 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).

   Tip: 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. Sample is ready for DNA extraction and purification.

4.2 DNA extraction

1. Pre-heat the incubator or water bath to 65 °C.

2. Prepare an extraction mix sufficient for all samples:

The extraction mix:	per sample (µL)	x 10 samples*
Extraction buffer**	150	1650
Proteinase K	6	66
2-mercaptoethanol or 5M 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 to room temperature before use.

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

Tip: 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 tubes and thoroughly re-suspend the pellet using a vortex.

5. Incubate the tubes for between 2 – 3 hours at 65 °C.

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

6. Vortex samples thoroughly and briefly centrifuge at maximum speed.

7. Pipette each DNA extract up and down 3 times using a fresh 1 mL filter-tip.

4.3 DNA purification

1. Take the DNA purification plate together with attached waste collection plate.
2. Add 350 µL equilibration solution to each well and stand at room temperature for 5 min.
3. Centrifuge for 1 min at 350 x *g*.
4. Discard the waste collection plate and place the DNA purification plate on the DNA collection plate.

5. Take the DNA pre-purification plate containing a white pellet. Transfer to each well 200 µL of DNA extract.
6. Carefully re-suspend the white pellet by pipetting up and down until it has fully mixed with the sample.
7. Centrifuge 1 min at 1,500 x *g*.
8. Transfer 100 µL of each sample from the DNA pre-purification plate to the equilibrated DNA purification plate. Be careful, try not to disturb the white pellet!
9. Stand the DNA purification plate for 3 min at room temperature.
10. Centrifuge 1 min at 700 x *g*.
11. The flow-through contains purified nematode DNA ready for Real-Time PCR or storage.

Note: We recommend storing the DNA between 4-8 °C for no more than several weeks. For longer-term storage keep at -20 °C or less. DNA purification plates should be sealed with the aluminium elution plate seal before storage.

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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