User Manual

Nematode DNA extraction kit

Scope: Individual nematodes and single 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 kit is designed to extract genomic DNA from individual nematodes and/or cysts. The obtained DNA extracts can be used directly for downstream applications like Real-Time PCR using one of ClearDetections' Real-Time PCR nematode identification kits (see www.cleardetections.com).

Note: To extract DNA from nematodes suspensions and/or multiple cysts, we recommend the 'ClearDetections kit Nematode DNA extraction for nematode suspensions and multiple cysts'.

2. Kit components, storage conditions and shelf life

Components	Description	Storage conditions
DNA extraction buffer (2x)	Bottle(s)	4-8°C
Proteinase K	Tube(s)	4-8°C

This kit should be stored at 4-8°C. If properly stored, the kit components can be used until the expiry date printed on the box.

3. Reagents and equipment to be supplied by the end-user

General

- Vortex
- Fume hood
- Centrifuge for tubes
- Temperature controlled incubator or water bath
- Pipettes and corresponding low adhesion* (e.g. siliconized) filter-tips for volumes of 5-1000 µL

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

For extraction of DNA from individual nematodes

- Low adhesion 1.5 ml tubes.

For extraction of DNA from individual 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.

4. Protocol for nematode DNA extraction

4.1 Sample preparation

Our protocol assumes that nematodes and/or cysts have already been isolated from sample materials, with unwanted debris such as soil aggregates or plant tissues removed.

Individual nematodes:

1. Add 50 µl deionized water to a clean 1.5 ml tube.
2. Transfer 1 individual nematode into the water using a nematode 'fishing or handling needle'.

   Note: Be careful not to touch the wall of the tube with the nematode and if possible check under the binocular if the transfer of the nematode into the water was successful.

Individual cysts:

1. Add 50 µl deionized water to a clean 1.5 ml tube.
2. Transfer 1 individual cyst into the water using e.g. a moistened fine painting brush.
3. Crush the cyst intensely using a clean pestle. Discard or disinfect the pestle.

   Note: See the instructional video "How to crush nematode cysts" published on www.youtube.com, demonstrating how you easily make your own pestles from pipette tips and how to crush cysts correctly.

4.2. Protocol for nematode DNA extraction

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

2. Prepare an extraction mix sufficient for all samples:

	per sample (µl)	x 10 samples*
2x extraction buffer **	50	550
Proteinase K	2	22
2-mercaptoethanol	0.5	5.5

   * 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 50 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. Centrifuge 30 seconds at 15000 g.

5. Incubate the tubes 30 minutes at 65°C with regular shaking.

   Note: Use the thermomixer set at 800 rpm or vortex samples thoroughly every 10 minutes and place them back in the incubator.

6. Take the tubes from the incubator or water bath and increase the temperature to 95°C.

7. Incubate the tubes 5 minutes at 95°C.

8. Centrifuge 30 seconds at 15000 g at room temperature.

9. The obtained DNA extract is ready for downstream application or storage.

   Note: If using the ClearDetections Real-Time PCR identification kit, dilute the crude DNA extract 5 fold (for individual nematodes) or 100 fold (for individual cysts). 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.

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 designed for general laboratory and research use only.

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