

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.



Nematode DNA extraction for individual nematodes and single cysts

EX-N-B-SNDE

For general laboratory and research use only

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 (RT-N-D/W-XXXX), see www.cleardetections.com.

Note: To extract DNA from nematode suspensions and/or multiple cysts, we recommend the 'ClearDetections Nematode DNA extraction & purification kit for nematode suspensions and multiple cysts' (EX-N-T/P-NDEP).

We recommend 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_SIN	4-8°C
Proteinase K	PK_SIN	4-8°C

Reagents and equipment to be supplied by the user

- Vortex
- Fume hood
- Centrifuge for tubes
- Temperature controlled incubator/heat block or water bath
- Pipettes and corresponding low adhesion* (e.g. siliconized) filter-tips for volumes of 5-1000 µL
- Low adhesion 1.5 mL microcentrifuge tubes
- Pestle (for crushing cysts)
- Nuclease free water
- β-Mercaptoethanol (2-Mercaptoethanol) or 5.0 M Dithiothreitol (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

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. 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).

Individual nematodes:

1. Add 50 µl deionized water to a clean low adhesion 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.

3. The sample is now ready for DNA extraction.

Single cysts:

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

4. The sample is now ready for DNA extraction.

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.

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:

Extraction mix	Per sample (µl)	x 10 samples*
Extraction buffer 2x**	50	550
Proteinase K	2	22
2-Mercaptoethanol /DTT	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 down 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 high speed, to make sure that the extraction mix and the nematode are at the bottom of the tube, in contact with each other.
5. Incubate the tubes 30 minutes at 65°C, and briefly vortex every 10 minutes.

Note: Alternatively, you can use a thermomixer set at 800 rpm.

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 high speed at room temperature, to collect the sample at the bottom of the tube.
9. Transfer the supernatant to a new tube.
10. The obtained DNA extract is ready for downstream application or storage.

Note: We recommend storing the DNA between 4-8 °C for no more than several days. For long-term storage keep at -20 °C or less.

Note: Before continuing with diagnostics, consider to dilute the DNA extract. If using the ClearDetections Real-Time PCR diagnostic kit, we recommend to dilute the crude DNA extract approximately 5 fold (for individual nematodes) or 100 fold (for individual cysts).