User Manual

Nematode DNA detection kit

Scope: All nematode species ('non-specific, general nematode DNA')

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 Real-Time PCR Nematode identification and detection kit offers a simple, rapid and reliable assay for the detection of DNA from any nematode species. This assay will accurately diagnose the presence of any nematode DNA obtained from a nematode suspension, a mixed nematode population or from an individual nematode/cyst. We recommend to use this 'General nematode assay' to confirm the presence of 'nematode DNA' in every DNA sample analyzed for the presence of a specific nematode species.

Note: Poor quality DNA will affect the results of this assay. The performance of each ClearDetections Real-Time PCR nematode identification and detection assay has been optimized for use with the ClearDetections Nematode DNA extraction & purification kit. We strongly recommend the use of this kit.

2. Kit components, storage conditions and shelf life

Components	Storage
ClearDetections'PCR mix	Room temp*
General nematode primer set	-20˚C
Positive amplification control (PAC)	Room temp*
Resuspension buffer	Room temp

* Supplied as lyophilized reagents. Should be stored in their original packaging at room temperature in a dry environment. After resuspension, store at -20 ˚C until their next use.
**During the shipment Nematode species specific DNA primer set(s) are stable at room temperature. After arrival store at -20 ˚C.
If properly stored, the kit components can be used until the expiry date printed on the box.

Note: The Positive amplification control (PAC) supplied consists of plasmid DNA in which the region targeted by the correspondent specific primer set has been inserted in a vector.

3. Additional reagents and equipment needed

- Centrifuge for either microcentrifuge tubes or for 96-well plates
- Pipettes and corresponding filter-tips for volumes of 5-1000 µl
- Microcentrifuge tubes or 96-wells plates for DNA dilution
- PCR plates & seals or PCR tubes
- Real-Time PCR machine*
- Nuclease free water
- Vortex

*Passive reference dye:
The ClearDetections PCR mixes do not contain a passive reference dye. If applicable, please deactivate any passive reference dye option in your Real-Time PCR software. Feel free to contact us for advice and support if this situation applies to you.

4. Detecting nematode DNA in samples by Real-Time PCR

4.1 Preparation of DNA samples and reagents for Real-Time PCR

Diluting DNA extracts is an **essential step** to further reduce the presence of compounds that inhibit Real-Time PCR. Traces of inhibitors are frequently encountered in materials from which nematodes are isolated, including diseased plant tissues and peaty soils.

Diluting each DNA extract **20 fold** (*e.g.*, 1 µL DNA in 19 µL nuclease free water) will normally ensure proper Real-Time PCR performance. Rarely, further dilutions are necessary if the DNA extract contains a high concentration of inhibitors (see Troubleshooting).

Resuspension of the Real-Time PCR Components

1. Gently tap the vial containing the ClearDetections PCR Mix, to settle any contents that may have moved during shipping.
2. Unscrew the cap of ClearDetections PCR mix vial and discard its rubber stopper.
3. Add 680 µL of resuspension buffer.
4. Incubate 5 mins at room temperature.
5. Add 105 µL of General nematode primer set into vial containing the ClearDetections PCR mix and mix gently by pipetting the content of vial up and down 5 times.

   Note: Mark the vial once the General nematode primer set is added.
6. Unscrew the cap of the PAC tube.
7. Add 300 µL of resuspension buffer.
8. Incubate 5 mins at room temperature.
9. Mix by vortexing and spin down the content of the tube.

   Note: Failing to properly mix the components will result in poor assay performance.
10. Proceed with the Real-Time PCR assay.

Tip: We recommend making aliquots of all resuspended reagents in order to prevent accidental contamination of master stocks.

4.2 General nematode DNA Real-Time PCR control

The presence of nematode DNA in a sample can be checked using the General Nematode DNA Real-Time PCR assay. Failure to detect nematode DNA using this Real-Time PCR assay indicates that there is either no nematode DNA present in the sample, or that the sample contains compounds that inhibit Real-Time PCR.

1. Design the layout of your Real-Time PCR experimental plate: one well for each sample to be screened for nematode DNA plus the two wells for the following controls:

   - Negative amplification control (NAC)
   - Positive amplification control (PAC)

2. Vortex the ClearDetections PCR mix vial containing General Nematode primer set for 1 – 2 sec.

3. Pipette 15 µL into each well.

4. Pipette 5 µL PAC into the PAC well.

   Take great care to avoid contaminating neighbouring wells.

5. Pipette 5 µL of nuclease free water into the NAC well.

6. Pipette 5 µL of each diluted DNA sample into its designated well.

   Note: the final volume in each well should be 20 µL.

7. Seal the plate and centrifuge for 1 min at maximum speed.

   No liquids should remain in the lid or sides of each well.

8. Transfer plate to the Real-Time PCR machine and start the run using the following settings:

	Step	Time	Temperature
Enzyme activation		3 min	95°C
Amplification (35 cycles)	DNA denaturation	10 sec	95°C
	Primer annealing	60 sec	63°C
	Primer extension*	30 sec	72°C
Melt curve*		0.2 - 0.5°C steps	72°C → 95°C

* Measure the fluorescent signal, using the FAM or SYBR/FAM channel, after every cycle and after every temperature increment of the PCR melt curve.

Run times, temperatures and volumes have been strictly optimized and must not be altered.

5. Interpretation of results

Quick interpretation scheme

1) Check Cq values in the PAC and NAC wells

Well	Signal Strength (Cq)	Interpretation
PAC	25 or less	Pass
	Above 25 (or no Cq)	Fail*
NAC	No Cq	Pass
	Less than 35	Fail*

If the PACs and NACs results pass

2) Check Cq values in the sample wells

Well	Signal strength (Cq)	Interpretation
DNA Sample	35 or less	Nematode DNA detected
	No signal	Nematode DNA not detected*

* See Troubleshooting

Explanation

Positive amplification control (PAC) well
A signal from the PAC well should have a Cq value of 25 or less if the assay was performed correctly.

Negative amplification control (NAC) well
A signal from the NAC well indicates contamination of the general nematode Real-Time PCR mix or NAC well by nematode DNA.

Sample wells
A signal from the general nematode Real-Time PCR indicates that nematode DNA was present in the sample. The absence of a signal indicates that there was no nematode DNA in the sample.

Remember: The lower the Cq value, the stronger the signal!

6. Troubleshooting

- Failed PAC (Cq above 25 or no Cq)

  Solution:
  > The assay should be repeated, ensuring that each protocol step is carefully performed.

- Failed NAC (Cq of 35 or less)

  The Real-Time PCR mix or NAC well was contaminated with nematode DNA, most likely from a sample, or the PAC.

  Solution:
  > All sample data must be ignored and the assay must be repeated.
  > Possible sources of contamination should be identified and removed.

- No general nematode signal detected in the sample wells

  Assuming nematodes were present in the sample material, failure to detect a general nematode DNA signal might be caused by inhibitors or a failed DNA extraction.

  - *Inhibition*

  A 20-fold dilution factor is usually sufficient to remove inhibitors from DNA extracted and purified from nematode suspensions. If detection is unsuccessful with the 20-fold dilution, additional dilution might be necessary.

  Solution:
  > Perform an additional 10-fold dilution and repeat the Real-Time PCR for the failed samples.

  - *Failed DNA preparation*.

  A problem occurred during the extraction or purification of nematode DNA.

  Solution:
  > Repeat the DNA extraction and purification using the ClearDetections nematode DNA extraction & purification kit.

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

7. 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.

For the legal notices & disclaimer see website, www.cleardetections.com, or contact ClearDetections at info@cleardetections.com