Nematode Staining Solution (Polychrome Blue Method)

N1510447

Storage Room temperature.
Shipping Regular transportation.

Introduction

Live nematode individuals can be obtained from infected tissues through methods such as direct picking with a dissecting needle, Baermann funnel separation, or homogenization of infected tissues. For the general observation of the morphology and structure of common nematodes for identification, temporary slides can be prepared using live nematodes. For further detailed observation and identification of their taxonomic characteristics, it is necessary to kill and fix the live nematodes; staining is sometimes required to prepare permanent slide specimens. If it is necessary to observe the interaction between nematodes and host tissues, paraffin sections of host and nematode tissues also need to be prepared.

Aladdin Nematode Staining Solution (Polychrome Blue Method) is mainly composed of methylene blue, ethanol, glycerol, etc. Nematodes are killed by heating and fixed, followed by staining with polychrome blue before observation. The staining principle is based on the differences in hydrogen ion concentration and physiological state of various internal organs of nematodes during fixation. This method can be well applied to the study of the structure of different organs of nematodes. This reagent is only used in the field of scientific research and is not suitable for clinical diagnosis or other purposes.

Product Components and Storage Conditions:

N1510447	Component	50mL	Storage
N1510447A	Nematode Dehydrant I	25mL	RT
N1510447B	Nematode Dehydrant II	25mL	RT
N1510447C	Polychrome Blue Staining Solution	5mL	RT

Self-prepared Materials:

1. Distilled water, 4% formalin fixative.
2. Glass slides, microscope, alcohol lamp, water bath, incubator.

Operating Procedures (For Reference Only):

1. Killing

The heat-fixation method is generally adopted. First, place a drop of distilled water on a glass

slide, pick a portion of nematodes onto the slide, and heat the slide over an alcohol lamp for 5–6 seconds while rotating it continuously. Observe the nematodes under a microscope at all times; stop heating immediately when the nematodes twist and then straighten suddenly, otherwise their internal organs will be damaged. Alternatively, place the nematodes in a drop of water and add a drop of hot 0.5% acetic acid to kill them. Another option is to use 0.1% iodine solution (mix equal volumes of nematode suspension and iodine solution, and the nematodes will be killed within a few seconds).

2. Fixation

Use TAF fixative or 4% formalin fixative.

3. Dehydration (Optional)

Place the fixed nematodes in a small glass dish containing 0.5 mL of nematode dehydrant I. Put the glass dish in a sealed container filled with excess 95% ethanol, and incubate in a 35–40℃ incubator for more than 12 hours. Add a small amount of nematode dehydrant to the glass dish, then place the dish in a semi-sealed petri dish and put it in a 40℃ incubator. After the ethanol in the dehydrant evaporates completely (usually taking 2–3 hours), transfer the treated nematodes into anhydrous glycerol for long-term preservation.

4. Staining

Directly transfer the fixed nematodes into a centrifuge tube containing 3 mL of distilled water and 3–5 drops of polychrome blue staining solution. Heat the tube in a 55–60℃ water bath for 3–5 minutes to stain the nematodes.

5. Staining Evaluation

Pick several nematodes and observe them under a magnifying glass or microscope. The staining is sufficient if the nematodes are uniformly stained dark purple and their internal organs are almost invisible. If there are still unstained gaps or uneven staining, extend the staining time appropriately.

6. Microscopic Examination

Place the stained nematodes in glycerol, cover with a coverslip, and observe under a microscope.

Staining Results:

Reproductive organs, oogonia, spermatogonia	Blue-purple
Cell nucleus	Light red
Chromosome	Blue–blue-purple
Intestine	Green (variable)
Other organs or cells	Dark purple or dark blue

Precautions:

1. Glass slides should be clean and free of oil stains.
2. Stained nematode specimens stored in anhydrous glycerol can be preserved for 2–5

months, after which they will gradually fade.

3. Generally, nematodes will be stained blue-purple, but not all morphological characteristics can be visualized by this staining method.
4. Avoid using high-temperature flames for fixation.
5. For your safety and health, please wear a lab coat and disposable gloves during operation.
6. Please use the reagent as soon as possible after opening to prevent affecting the results of subsequent experiments.