

Pythium spp. High-Sensitivity Field Nucleic Acid Test Kit — Instructions for Use (IFU)

Product Name

Generic name: Pythium spp. High-Sensitivity Field Nucleic Acid Test Kit

Package Specification

16 tests/box

Intended Use

This kit amplifies nucleic acid sequences specific to Pythium spp. and uses directional signal amplification technology to achieve high-sensitivity detection of the target nucleic acid. Applicable sample types: corn stalks and corn seeds.

Test Principle

This kit amplifies nucleic acid sequences specific to Pythium spp. and uses directional signal amplification technology to achieve high-sensitivity detection of the target nucleic acid.

Main Components

Table 1. Main components

No.	Component	Main ingredients	Specification	Qty.
1	Sample soaking solution	Buffer	60 mL/bottle	1
2	Zip bags	—	16 pcs/bag	1
3	Pythium spp. enzyme pellet	Primers, probe, endonuclease, deoxyribonucleoside triphosphates, polymerase	16 tests/pack	1
4	Pythium spp. positive control	Target plasmid containing the target fragment	280 µL/tube	1
5	Negative control	Nuclease-free water	280 µL/tube	1
6	Lysis solution	Buffer, resin	16 tubes/bag	1

Storage Conditions and Shelf Life

Store at 2–8°C. Shelf life: 12 months.

Sample Handling

1.

Gently rinse the sample under running water to remove soil and surface debris.
2.

Using sterile scissors or a scalpel, cut the basal portion of the corn stalk and place it in a zip bag. Clearly label each sample with its source, collection date, and other relevant information.
3.

Keep samples at an appropriate low temperature using ice packs or a cooler to prevent deterioration.

Sample Preparation

1.

Before starting the test, turn on the metal dry bath, set it to 95°C, and begin heating. Remove the sample soaking solution and zip bags, and prepare disposable gloves and scissors.
2.

While wearing disposable gloves, place an appropriate amount of seed (approximately 5 seeds recommended) or corn stem-base material from three locations into a zip bag and label it.
3.

Change disposable gloves. Add enough sample soaking solution to submerge the sample, then shake the zip bag up and down 5 times.

Note: Change disposable gloves before preparing each sample to avoid cross-contamination between samples.

Note: If the seeds are coated, thoroughly wash off the coating with clean water before placing them in the zip bag.

Note: Do not rub the zip bag forcefully; avoid damaging the bag.

Note: Do not over-rub the stem-base material into a slurry. If red, yellow, green, or other abnormal colors appear after rubbing, prepare a new sample to ensure the accuracy of subsequent testing.

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Nucleic Acid Extraction

1. Remove the required number of lysis-solution tubes. Flick each tube downward forcefully 3 times to collect the liquid at the bottom, and label the tubes.
2. Transfer 50 μ L of supernatant from the zip bag into a lysis-solution tube and close the cap tightly.
3. Mix the lysis-solution tube forcefully up and down 5 times, then flick it downward forcefully 3 times to collect the liquid at the bottom. Incubate in a metal dry bath that has reached 95°C for 10 min. Remove and allow to cool to obtain the nucleic acid solution for testing. If testing cannot be performed immediately, store at 4°C for no more than 1 h.

Note: When withdrawing liquid from the zip bag, do not allow the pipette to touch the inner wall of the bag and avoid aspirating solids.

Test Procedure

1. Remove and cut off the required number of enzyme-pellet tubes. Along the wall of separate tubes, add 50 μ L of negative control, nucleic acid solution for testing (use the supernatant and avoid solids at the bottom), and positive control, respectively.
2. Close the tube caps tightly. Hold both ends of each tube between the thumb and index finger, mix forcefully up and down 10 times, and then flick downward forcefully 3 times to collect the liquid at the bottom. Visually confirm that the enzyme pellet is completely dissolved, the liquid level is uniform, and no bubbles remain at the bottom; then place the tubes in the palm-sized fluorescence detector for testing.

Note: Manual mixing is a critical step and must be performed thoroughly. Before starting the test, make sure the enzyme-pellet tube caps and the lid of the palm-sized fluorescence detector are closed tightly.

Result Interpretation

At the end of the test program, the palm-sized fluorescence detector automatically analyzes and determines the result.

Table 2. Result interpretation for the palm-sized fluorescence detector

Assessment	TT display	Displayed result
Negative control	—	NC
Positive control	TT $\leq$ 10	PC
Positive sample	TT $\leq$ 20	Positive
Negative sample	—	Negative

Equipment and Consumables Required but Not Provided

1. Personal protective equipment (disposable gloves, laboratory coat, protective eyewear, etc.).
2. Timer.
3. Liquid-waste container.

Precautions

1. Do not use expired reagents.
2. Perform the entire procedure in a nuclease-free environment.
3. Wear disposable gloves during operation.
4. Use filtered pipette tips to prevent contamination of the pipette.
5. All sample tubes, centrifuge tubes, tips, reagents, and test tubes are single-use and must not be reused.
6. Do not mix reagents from different product lots.

IFU Approval and Revision Date

August 21, 2026