

ICS 65.020

NY

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**Detection and identification method of Cucumber green mottle
mosaic virus
(English Translation)**

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Table of Contents

Foreword...	3
1 Scope...	5
2 Normative references...	5
3 Terms and definitions...	5
4 Principles...	5
5 Reagents and materials...	6
6 Instruments and utensils...	6
7 Sampling...	6
8 Testing...	6
9 Results judgement and reporting...	7
10 Sample processing...	8
11 Archive preservation...	8
Appendix A (Informative) Reagent preparation method...	9

1 Scope

This document specifies the sampling methods, quarantine detection, result determination and reporting, sample processing, file preservation of cucumber mottle mosaic virus.

This document is applicable to the quarantine detection and identification of cucumber mottle mosaic virus, in agricultural plant quarantine.

2 Normative references

The provisions in following documents become the provisions of this Standard through reference in this Standard. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this standard.

GB 15569 Quarantine protocol for the movement of agricultural plants and plant products

GB/T 28071 Detection and identification of cucumber green mottle mosaic virus

3 Terms and definitions

There are no terms and definitions, that need to be defined in this document.

4 Principles

Cucumber green mottle mosaic virus (CGMMV) belongs to the genus Tobacco mosaic virus, which spreads with seeds, scions, rootstocks. The important basis for the virus quarantine detection and identification is typical symptoms and other rapid diagnosis.

It is combined with the detection results of virus double antibody sandwich enzyme-linked immunosorbent (ELISA) or the results of reverse transcription polymerase chain reaction (RT-PCR), for comprehensive judgement.

5 Reagents and materials

Sodium carbonate (Na_2CO_3), sodium bicarbonate (NaHCO_3), anhydrous disodium hydrogen phosphate (Na_2HPO_4), anhydrous potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride (MgCl_2), anhydrous sodium sulfite (Na_2SO_3), sodium hydroxide (NaOH), sodium azide (NaN_3), ethanol ($\text{CH}_3\text{CH}_2\text{OH}$), disodium p-nitrophenylphosphonate (PNPP), diethyl pyrocarbonate (DEPC), deionized water, commercially available antibodies, commercially available enzyme-labeled antibodies, commercially available PCR reagents, commercially available Trizol reagents, etc. The ratio of reagents, which are required in the testing, is as shown in Appendix A.

All reagents are of analytical pure.

6 Instruments and utensils

PCR instrument, desktop high-speed refrigerated centrifuge, spectrophotometer, electronic balance, enzyme-linked plate, enzyme-linked reader, enzyme-free plate machine, micropipette, water bath, electrophoresis instrument and horizontal electrophoresis tank, gel imaging system, incubators, refrigerators, etc.

7.1 Seeds

The sampling quantity and method shall be carried out, according to the provisions of GB 15569. The sampling shall be sufficient and representative.

7.2 Leaves and fruits

Randomly select the diseased or suspected diseased leaves or fruits, mainly young leaves and fruits. Each fruit sample shall be not less than 200 g; the leaves shall be not less than 10 g. They shall be sealed in a Ziplock bag and stored at 4 °C, indicating the information, such as the name of the plant, collection time, place, collector.

8.1 Symptom inspection

Check the leaves, stems, fruits, for comparison with typical symptoms of cucumber green mottle mosaic virus disease (see Appendix B).

8.2 Rapid diagnosis

See Appendix C, for rapid diagnostic test strips or diagnostic kits. If using commercially available test strips or kits, follow the instructions to operate.

8.3 Enzyme linked immunosorbent assay (ELISA)

See Appendix D, for enzyme linked immunosorbent assay of cucumber green mottle mosaic virus. If a commercially available kit is used, follow the instructions to operate.

8.4 Reverse transcription polymerase chain reaction detection (RT-PCR)

Refer to Appendix E, for the reverse transcription polymerase chain reaction detection (RT-PCR) of cucumber green mottle mosaic virus. If using a commercially available kit, follow the instructions to operate.

9.1.1 Typical symptoms

If the fruit is diseased and has typical symptoms, it can be initially judged.

9.1.2 Rapid diagnostic results

If the test result of rapid diagnostic test strip or diagnostic kit is positive, it can be judged as highly suspected.

9.1.3 Enzyme linked immunosorbent assay (ELISA)

Under the premise that the OD value of the negative control optical density value is $\leq$

0.1, AND positive control's OD value is greater than 2 times the negative control's OD value, if the sample hole's OD value is greater than 2 times the negative control's OD value, it is determined as a positive reaction, that is, the sample has cucumber green mottle mosaic virus.

9.1.4 Reverse transcription polymerase chain reaction detection (RT-PCR)

Observe the electrophoresis results. On the premise that the positive control has a band at 654 bp, whilst the blank and negative controls have no band, if the sample to be tested has a band at 654 bp, THEN, it is judged as a positive reaction, that is, the sample has cucumber green mottle mosaic virus.

9.1.5 Result report

Fill in the identification results, in the plant pest sample identification report, in Appendix F.

Appendix A (Informative)

Reagent preparation method

A.1 Coating buffer

Take 1.59 g of Na_2CO_3 , 2.93 g of NaHCO_3 , 0.2 g of NaN_3 . Use deionized water to dilute it to 1000 mL. Adjust the pH to 9.6. Store it at 4 °C.

A.2 Washing buffer

Take 1.15 g of Na_2HPO_4 , 0.2 g of KH_2PO_4 , 8.0 g of NaCl , 0.2 g of KCl , 0.5 g of Tween-20. Use deionized water to dilute it to 1000 mL. Adjust pH to 7.4. Store it at room temperature.

A.3 Extraction buffer

Take 2.0 g of egg white powder, 20.0 g of polyvinylpyrrolidone (PVP, K30) (molecular weight 44000 ~ 54000), 1.3 g of Na_2SO_3 , 0.2 g of NaN_3 , 20.0 g of Tween-20. Dissolve it in 1000 mL of 1XPBST. Adjust pH to 7.4. Store it at 4 °C.

A.4 Enzyme-labeled antibody buffer

Take 2.0 g of bovine serum albumin, 10.0 g of polyvinylpyrrolidone (PVP, K30), 0.2 g of NaN_3 . Dissolve it in 1000 mL of 1XPBST. Adjust to pH 7.4. Store it at 4 °C.

A.5 Substrate buffer

Take 97.0 mL of diethanolamine, 0.1 g of MgCl_2 , 0.2 g of NaN_3 . Use deionized water to dilute it to 800 mL. Adjust pH to 9.8. Store it at 4 °C.

Appendix B (Informative)

Basic information of cucumber green mottle mosaic virus

B.1 Host range

Watermelon, gourd, cucumber, pumpkin, gourd and other melon crops.