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| **VERSION:** 1.0 | **DATE:** 12/2012 |
| **PATHOGEN:** Cucumber Mosaic Virus (CMV) | |
| **HOST:** spinach (Spinacia oleracea) | |
| **COMMON NAME:** | |
| **METHOD:** Lcb 5.1 Seedling growout and ELISA (Yang et al., 1997) | |
| **METHOD CLASS:** STANDARD (A) | |
| **SAMPLE:** | |

**PROCEDURE:**

1. Germinate seeds from suspected infected plants in large flats containing Redi-Earth 3CF potting mixture (Grace Sierra, Milpitas, CA).

2. Grow plants in a growth chamber with a 10-h day at 21°C or in a greenhouse with a 9-11h day at temperatures ranging from 20° to 30°C. The plants need to be carefully monitored to ensure they remain free of aphids.

3. When seedlings reach the 5-leaf stage harvest ~1.5 g of mature leaf tissue.

4. Extract plant sap and dilute 1:10 with phosphate buffered saline-Tween buffer.

5. Use protein A sandwich indirect ELISA (PAS-ELISA) to detect CMV. (CMV polyclonal antiserum (1:10,000 in PBST)).

6. Add alkaline phosphatase substrate (p-nitrophenyl phosphate, di-sodium; Sigma Chemical Co., St. Louis).

7. To quantify the reaction, determine absorbance at 405 nm with a microplate reader (Cambridge Technology Inc., Watertown, MA).

8. A sample is considered positive for CMV infection when the mean absorbance of two replicates is three times greater than the absorbance of the uninfected plant controls.

**RECIPE:**

PBST
137 mM NaCl, 1.5 mM $K_2HPO_4$, 8 mM $Na_2HPO_4$, 2.7 mM KCl, 0.05% [vol/vol] Tween 20, pH 7.4

**REFERENCES:**

Yang, Y., Kim, K. S. and Anderson, E. 1997. Seed transmission of cucumber mosaic virus in spinach. Phytopathology. 87(9):924-931.