46

Assaying Levels of Plant Virus by ELISA

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1. Introduction

Enzyme-linked immunosorbent assay (ELISA), since its use with plants was described by Clark and Adams ***(1)***, has become the most popular method for detection of viruses in plants, because of its simplicity and wide applicability. Used mainly to confirm presence or absence of infection, it can be adapted to estimate concentration of an antigen, e.g., viral capsid protein, in plant sap. The procedure described in detail here is that for double antibody sandwich (DAS) ELISA with alkaline phosphatase-conjugated antibody, which suffices for most occasions. However, the principles outlined will apply equally well to indirect ELISA, using an antispecies conjugate, or to plate trapped antigen ELISA, should those variants be the method of choice for a specific viral protein.

The text is worded for those who already have, or can purchase, viral-specific IgG and enzyme-conjugated antibodies. Preparation of antibodies is described in Chapter 29 of this volume. Optimization of IgG and conjugated antibody dilutions is fully described by Hill ***(2)*** and Converse and Martin ***(3)***.

2. Materials

1. 96-Well flat-bottomed polystyrene or polyvinyl microtiter plates suitable for binding of IgG.
2. Viral protein-specific IgG for coating wells.
3. Viral protein-specific antibody conjugated to alkaline phosphatase.
4. Coating buffer: 0.1*M* carbonate buffer, adjusted to pH 9.6 (1.59 g Na_2CO_3, 2.93 g $NaHCO_3$ per L).
5. Sample buffer: Normally phosphate-buffered saline (PBS) pH 7.4: 8 g NaCl, 0.2 g KH_2PO_4, 2.9 g $Na_2HPO_4 \cdot 12\ H_2O$ per L, with the addition of 0.05% Tween-20 and 2% polyvinylpyrrolidone, mol wt 40,000.

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