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Western Analysis of Transgenic Plants

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1. Introduction

Western blotting combines the resolving power of protein electrophoresis and the specificity of immunology in a rapid and sensitive format for the identification of proteins in complex mixtures. Proteins resolved by electrophoresis are transferred to a solid support, which is normally nitrocellulose or polyvinylidene fluoride (PVDF [Millipore, Bedford, MA]). Immunological detection of proteins transferred to a solid support combines ease of handling with accessibility of antibodies to the immobilized protein. A primary antibody is bound to a specific antigen on the membrane, and this antibody is detected using a labeled high-affinity reporter (frequently an enzyme-linked antibody). This chapter describes a basic protocol for the Western blotting and detection of transgene expression products in plants (**Fig. 1**). An alternative procedure for the initial immunopurification and concentration of a transgene product is also described, based on the use of affinity-purified antibodies covalently attached to magnetic beads. Western blotting can be applied to any gel separation technique; however, one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) ***(1,2)*** is most commonly used.

2. Materials

1. Extraction buffer: 50 m*M* Tris-HCl, pH 8.0, 5 m*M* EDTA, 1X proteinase inhibitors (1 mg/mL each). The proteinase inhibitors (leupeptin [Sigma, St. Louis, MO], antipain [Sigma], and aprotinin [Sigma]) are stored together as a 1000X stock (1 μg/μL each) at –20°C.
2. SDS-PAGE (5X) sample buffer: 0.31*M* Tris-HCl, pH 6.8, 50% glycerol, 5% (w/v) SDS, 10% (v/v) β-mercaptoethanol, 0.1% bromophenol blue.

From: *Methods in Molecular Biology, Vol. 81: Plant Virology Protocols: From Virus Isolation to Transgenic Resistance*
Edited by: G. D. Foster and S. C. Taylor