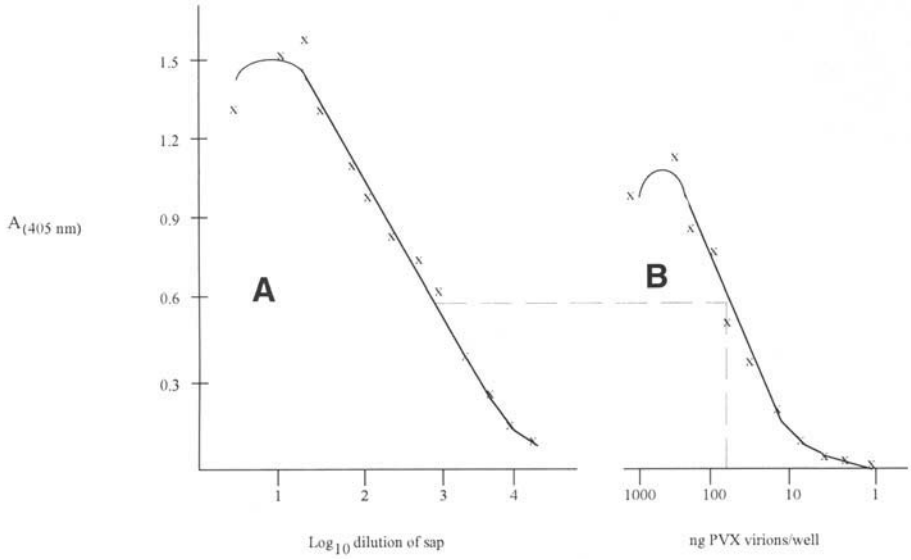

Fig. 1. Relationship between concentration of viral protein and absorbance in DAS-ELISA. **(A)** Diluted sap of PVX-infected leaves. **(B)** Purified PVX virions diluted in sample buffer.

common cause of elevated absorbance values in rows, columns, or single wells of microplates. Turning a microtiter plate through 180° and repeating the wash program is a simple way of ensuring that all wells are washed thoroughly when using a mechanized washer. Plant debris that settles on the floor of wells and is not dislodged during washing increases background color. Mechanical agitation of plates at the end of **step 4** of the ELISA procedure should overcome the problem. Even if using a plate-washing machine that will aspirate contents of wells, it is advisable to manually empty each plate after incubation of diluted sap and antibody-enzyme conjugate, and wipe dry the top surface, before washing with PBS-Tween. Errors caused by uneven background color development are more easily detected if there are at least duplicate wells of each sample on a microtiter plate.

2. Agents commonly reported to be effective in blocking uncoated reactive sites on the surfaces of wells are: 0.2–2% solution of powered nonfat milk, bovine serum albumin, ovalbumin, or gelatine diluted in PBS-Tween and incubated for 1–2 h at 25°C; 1 μg/mL polyvinyl alcohol (Sigma P8136) in PBS-Tween, incubated for 1 min at room temperature.
3. A plot of absorbance against Log_{10} antigen concentration in DAS ELISA will produce a sigmoid curve with depressed absorbance values at the very highest concentrations of antigen (**Fig. 1**). Accurate quantitative analysis is possible only in the median range of antigen concentration, where absorbance responds lin-