early to change in antigen concentration. Thus, when assaying concentration of viral protein in transgenic plants, a preliminary assay has to be run with serial dilutions of sap from virus inoculated untransformed plants of the parent genotype inoculated and grown in identical conditions to determine the range of dilutions that lie within the linear phase of the sigmoid curve. A similar assay is needed to quantify the response to concentrations of purified virions or viral protein (**Fig. 1B**). In the linear phase, both curves should have equal slopes.

The dilution of sap chosen for assay of transgenic plants should produce absorbance values that fall within the linear phase of the associated calibration assay of purified viral protein. If it is anticipated that transgenic plants will have a lower virus content than the parent genotype, then a dilution at the upper end of the linear phase would be appropriate (e.g., 1:100 in the assay depicted in **Fig. 1A**). Two or more dilutions of sap of test samples may have to be included if the plant material to be assayed is expected to have a wide variation in viral concentration.

4. The distribution of samples on a microtiter plate is a matter of individual choice, often constrained to a degree by the software program in the colorimeter/plate reader used to measure absorbance. Each plate, though, should have wells to which only reagents have been added, and against which background absorbance the plate is blanked in a plate reader; wells with a range of concentrations of purified viral protein diluted in sample buffer; wells with sap from untransformed, virus-inoculated plants; wells with sap from untransformed, virus-free plants; wells with sap from virus-inoculated plants of each transgenic line; and wells with sap from virus-free plants of each transgenic line. Bunch et al. ***(4)*** have described in detail how allocation of wells on a 96-well microtiter plate between calibration and test samples influences accuracy and efficiency in quantitative ELISA. Their calculations are complex and need recalculation for every protocol, but, simplified, they show that (1) the calibration assay should contain at least four concentrations of viral protein, distributed over the linear phase of the absorbance curve; and that (2) all test samples should be included on each plate. It is more efficient to split replicates between plates rather than test samples.
5. Prior to reading color development, wipe the top surface of each plate to prevent substrate solution from contaminating the photocells in the reader.
6. Evaporation from wells during incubation of plates can be minimized by using proper plate covers or by simply placing an empty used plate on top and putting the stack of plates in a polythene bag.
7. If an indirect ELISA using an antispecies antibody–enzyme conjugate is required, then the second virus protein-specific antibody is incubated at **step 6** of the ELISA procedure, and, after washing, the antispecies conjugate is incubated in wells for 3–4 h at 25°C or 2–3 h at 37°C.
8. If plate-trapped antigen ELISA is necessary, determination of the optimum dilution of sap in buffer is still required as a prelude to the actual assay for viral protein.