

been modified with labels, often enzymes, which are attached by a simple conjugation process.

Before use in ELISA or blots, antibodies must be evaluated. It is desirable to test them for reactivity with other viruses, particularly closely related ones, for their reactions with healthy sap, and to optimize the dilutions to be used routinely. This should be done for each batch of antiserum (*see* **Notes 2** and **3**).

2. Materials

2.1. Buffers for Antibody Purification

Prepare solutions fresh and store at 4°C until required.

1. Phosphate-buffered saline (PBS), pH 7.4, 10X concentrate: 80.0 g NaCl, 2.0 g KH_2PO_4 , 29.0 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 2.0 g KCl.

Make up to 1 L with distilled water. The concentrate keeps well without the addition of sodium azide and can be diluted as required.

2.2. Media Required for Tissue Culture

1. Mixed thymocyte medium (MTM) (**6**): This is required as a supplement for cells after fusion and for cloning (*see* **Note 4**). It is prepared by culturing the thymocytes of two different strains of rat in medium containing 15% fetal bovine serum (FBS) (Sigma, Dorset, UK) (*see* **Note 5**) for 48 h. The medium is recovered from the cells by centrifugation and stored at -70°C until required.
2. HAT medium: Medium containing 15% FBS, hypoxanthine, aminopterin, and thymidine (HAT) (Gibco-BRL, Paisley, Scotland) is required for the selection of recombinant cells after cell fusion (*see* **Note 6**). It should be prepared in advance and stored at 4°C.
3. RPMI-1640 (Sigma): RPMI-1640 containing 10 and 15% FBS is also required for routine tissue culture.

2.3. Consumables and Chemicals for Antibody Purification/Modification

1. Centriprep-30 and Centricon-30 concentrator tubes available from Amicon.
2. Alkaline phosphatase enzyme suspension in ammonium sulfate available from Sigma; Type VII.
3. Mabtrap available from Pharmacia.

3. Methods

3.1. PAb Production

1. Mix 200–500 µg of purified virus (*see* **Notes 7** and **8**) with an appropriate adjuvant to a final volume of 0.5 mL. Inject intramuscularly into the hind leg of the rabbit or subcutaneously into the neck scruff.