

## Virus Purification Procedure

- Supernatants were harvested 72 h following infection of High Five™ cells with recombinant HIV-gag baculoviruses encoding the HIV group specific antigen (Pr55<sup>gag</sup>) and precleaned by low speed centrifugation at 300 g and room temperature for 5 minutes.
- Pr55<sup>gag</sup>-virus-like-particles and the baculoviruses were pelleted through a 30 % sucrose cushion by ultracentrifugation (Centrikon T-1160, Kontron; SW28 Rotor, Beckman; 2.5 h at 25,000 rpm, 16°C). The obtained pellets were dissolved in PBS without bivalent ions. The protein concentration of the preparation was measured by a Bradford-Protein-Assay (BIORAD, Munich).
- 500 µg protein of the preparation was given on a sucrose gradient (10% to 50%) and was centrifuged (Centrikon T-1160; SW41 Rotor, Beckman) for 2.5 h at 28,000 rpm and 16°C.
- Starting from the top of the gradient, 550 µl fractions were carefully removed.
- An 10 µl aliquot of each fraction was analyzed by western blotting and the Pr55<sup>Gag</sup> content of each fraction was determined. Respectively fraction 14, yielding the majority of the Pr55<sup>Gag</sup> protein, was used for the immunogold labelling.