

Figure 1. HEV71 VLP expression cassette [P1+IRES+3CD] and the pSN01 plasmid.

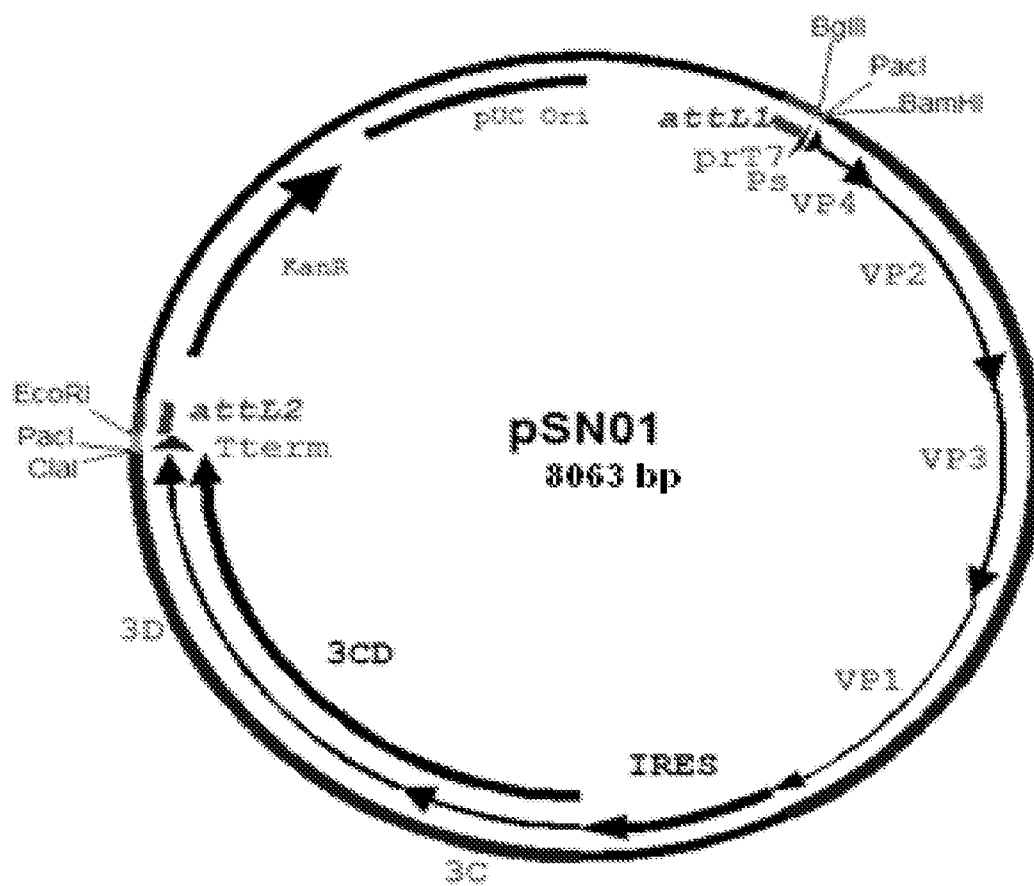
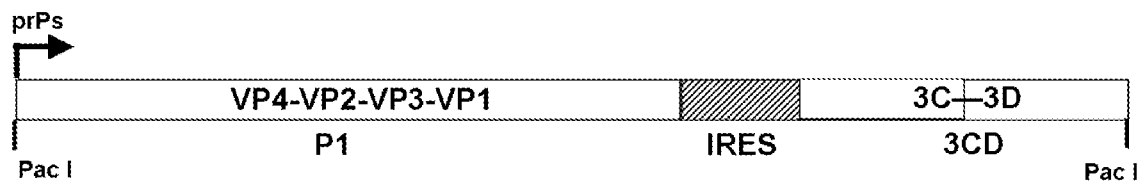


Figure 2. HEV71 VLP expression cassette [P1+IRES+3C] and the pSN03 plasmid.

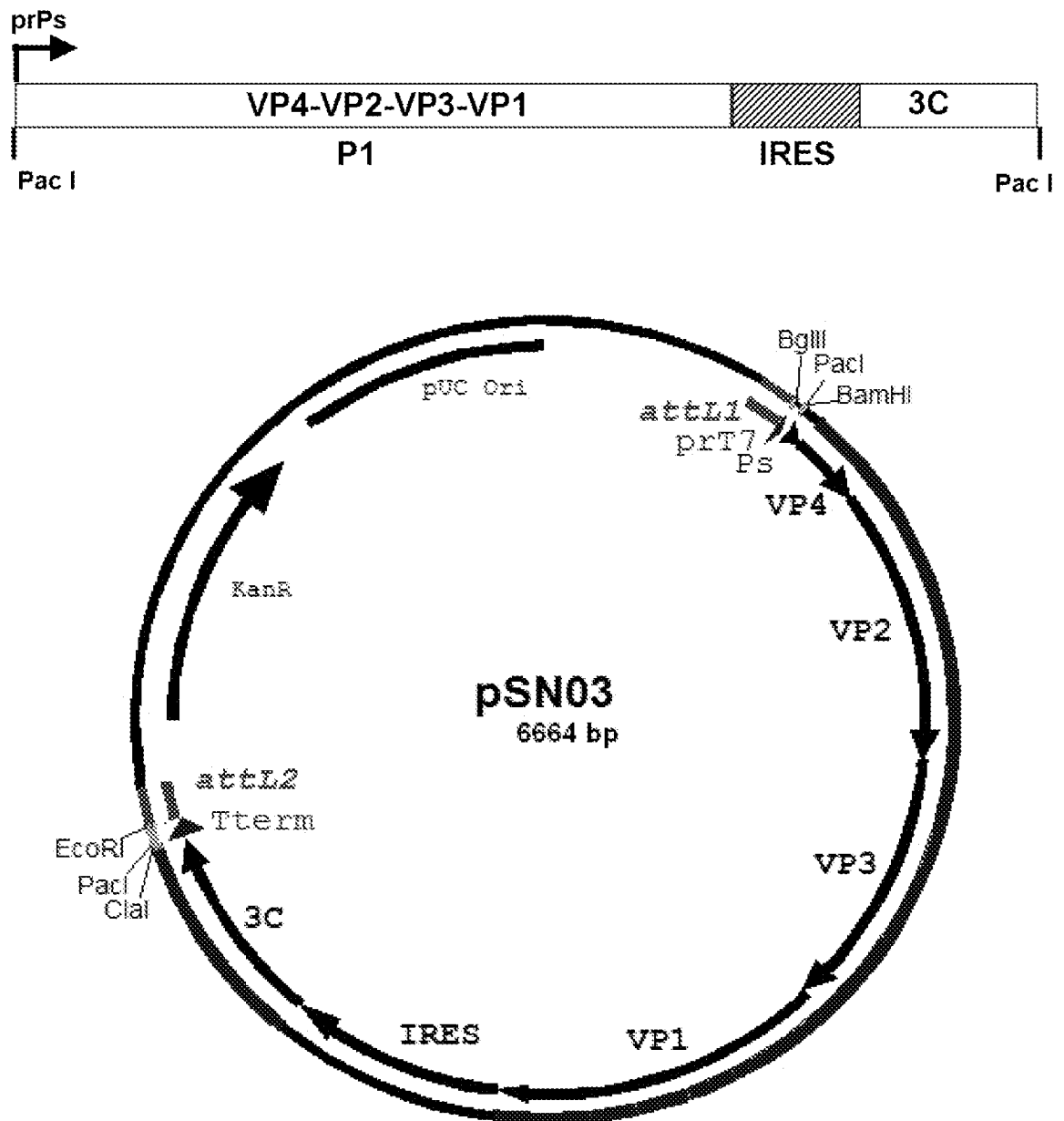


Figure 3. Expression of VP1 in supernatant of SN07 infected Sf9 cells.

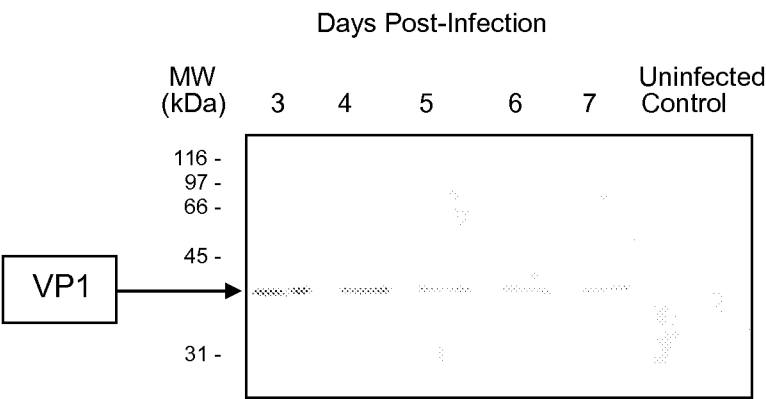


Figure 4. Processed VP1 in both the supernatants and the lysates.

Lanes 1 and 5: bacSN07p3

Lanes 2 and 6: bacSN08p3

Lanes 3 and 7: bacGUSd2

Lanes 4 and 8: Sf9 control

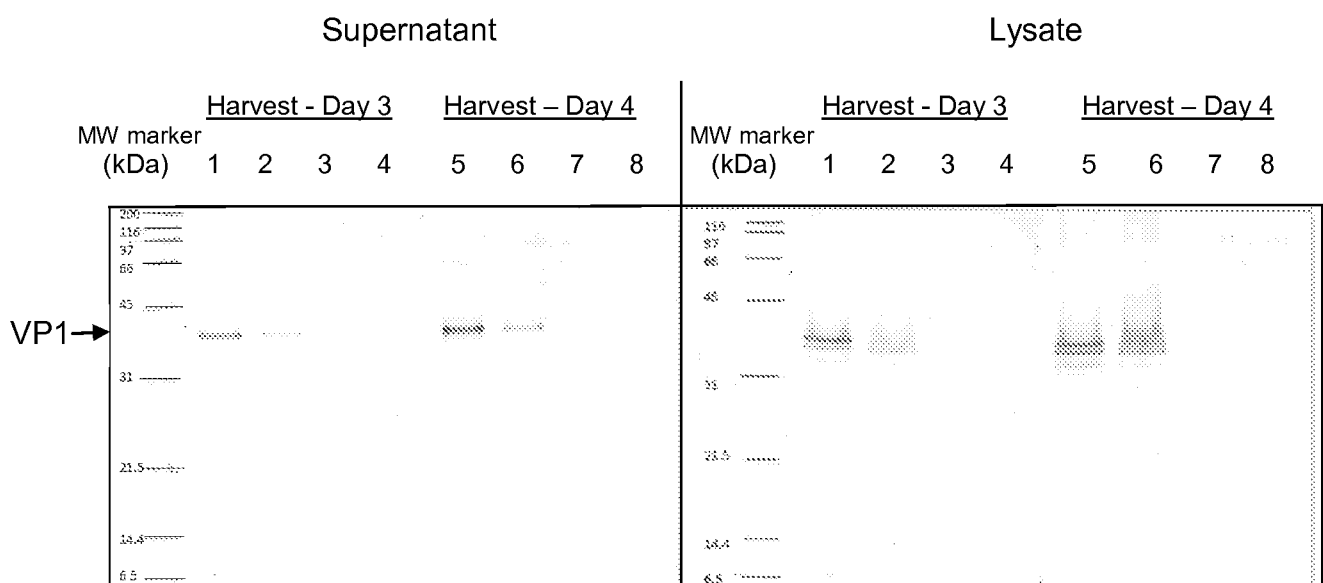


Figure 5. VP1 and VP0 are in the retentate after ultrafiltration over a 100kDa molecular weight cut off (MWCO) membrane.

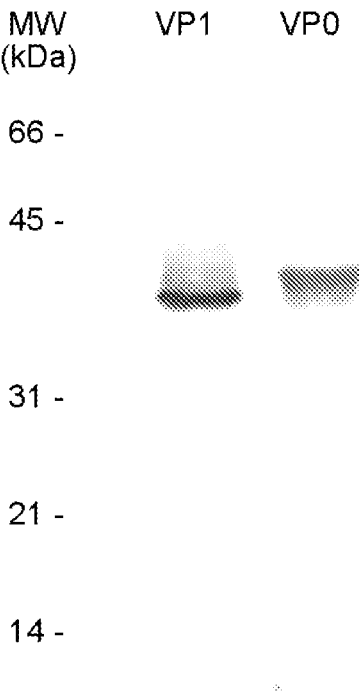


Figure 6.

FIRST PROOF OF CONCEPT OF OUR STRATEGY:  
TWO IMMUNIZATION SCHEDULES (4 mice per group).

The immunogen was prepared in Freund's Complete Adjuvant (FCA) or Freund's Incomplete Adjuvant (FIA) and administered via subcutaneous injection (sc) or intraperitoneal injection (ip).

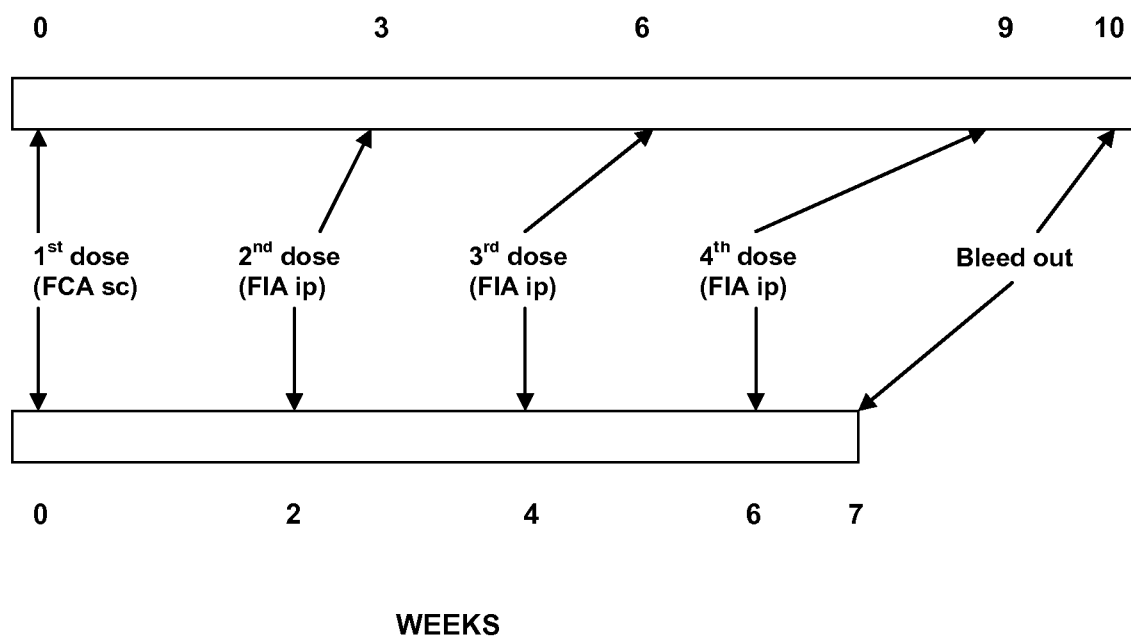


Figure 7. Immunoblots probed with rabbit polyclonal antisera against VP0 (arrow) at 3 different times of harvest.

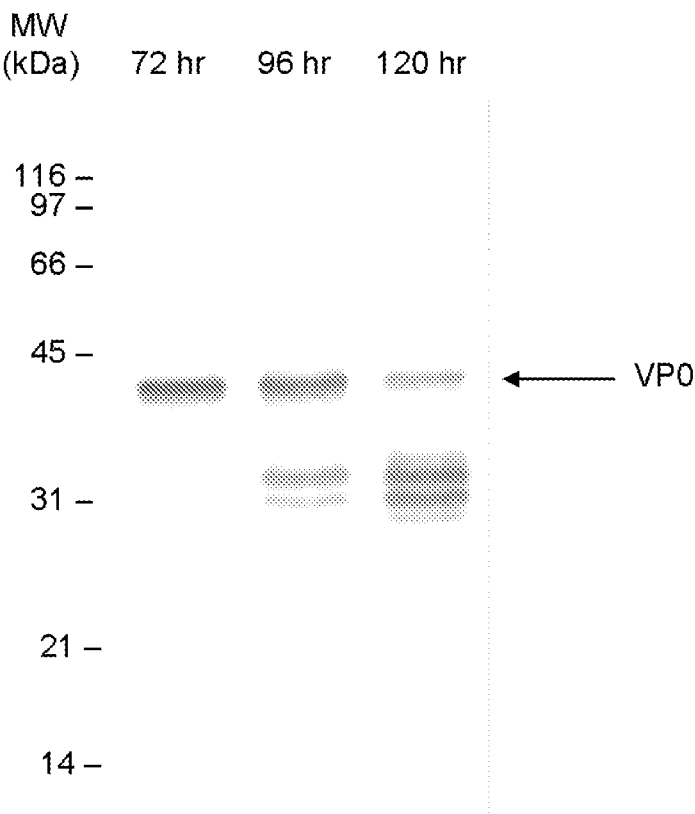


Figure 8. Pooled neutralizing sera from mice immunized with the oligomeric antigens in the supernatant of SN07 infected Sf9 cells have high titres against recombinant VP2 in ELISA.

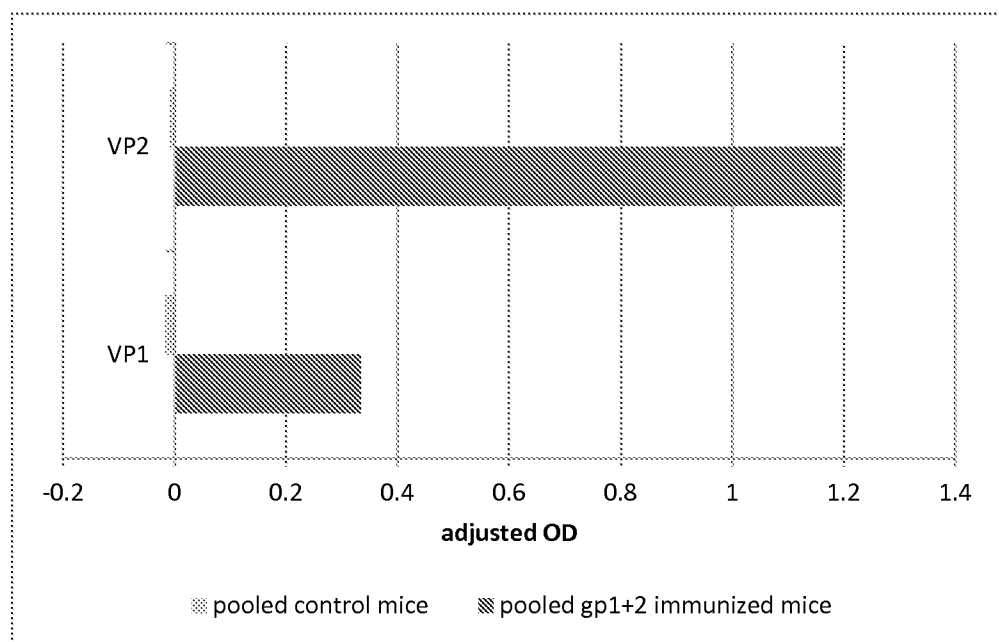
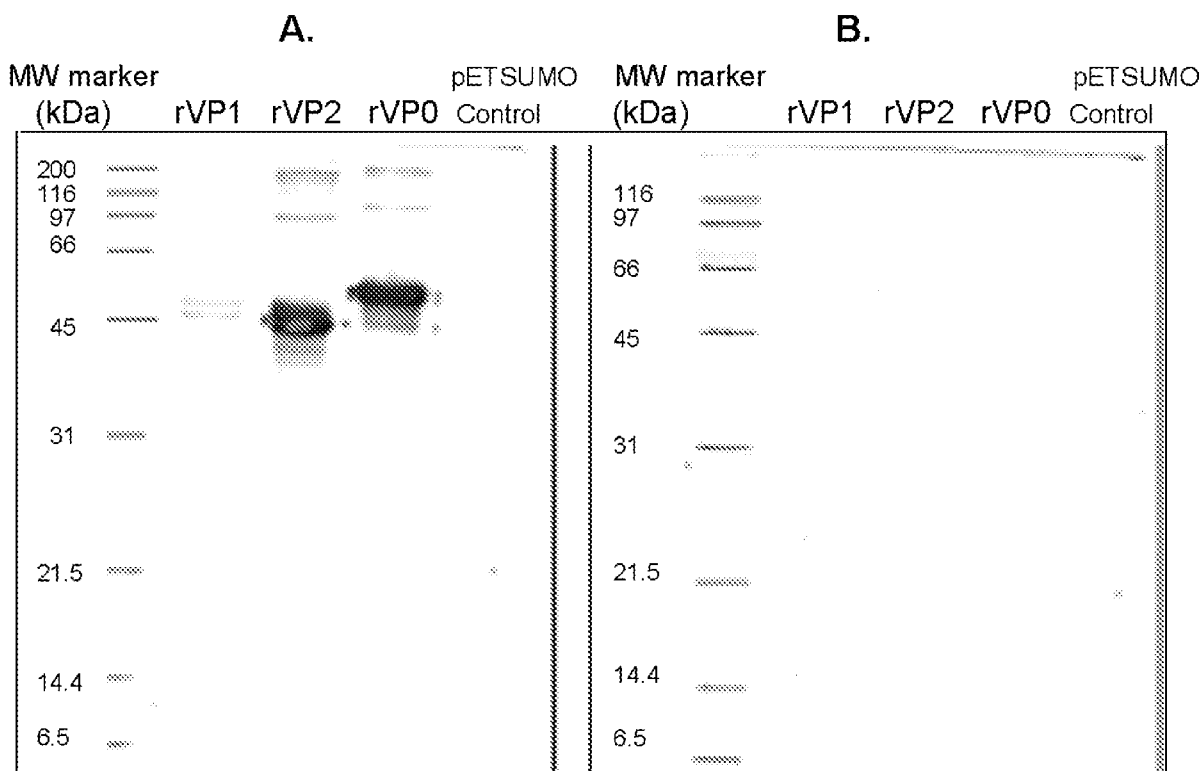


Figure 9. Pooled neutralizing sera from mice immunized with oligomeric antigens in the supernatant of SN07 infected Sf9 cells bind more strongly to VP2 and VP0 than to VP1.



A. Probed with mice serum anti-bacSN07p3 VLPs at 1/2000 dilution.

B. Probed with mice serum anti-SF9 control cells at 1/500 dilution.

Figure 10. IRES region of the EMCV genome (SEQ ID NO:1). The IRES region represents the EMCV genomic sequence in GenBank accession number AF113968.2 ; nucleotides 1666 to 2251.

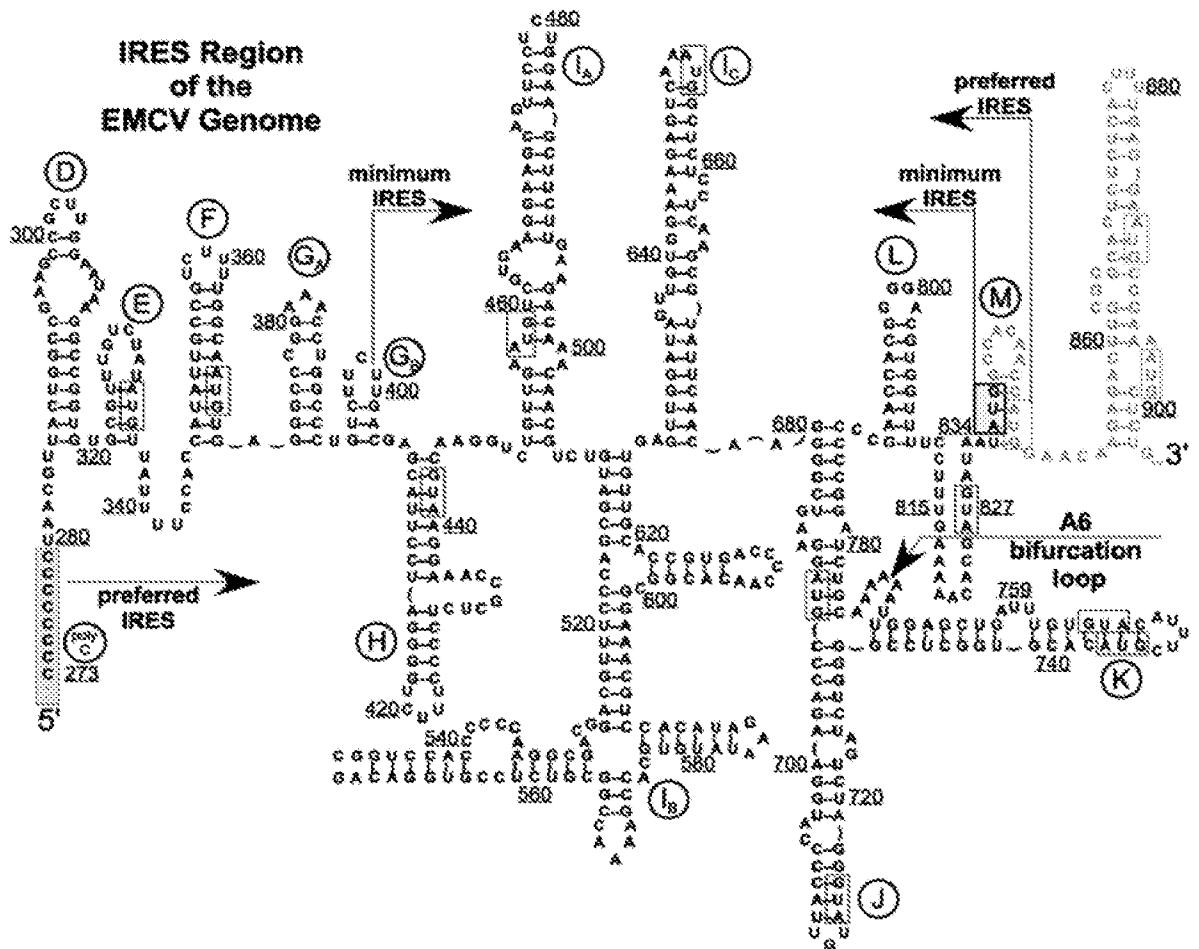


Figure 11. Out framing of the EMCV start codon with the 3CD protease coding sequence; Native EMCV IRES coding sequence (SEQ ID NO:2) is compared to a mutant EMCV IRES sequence coding sequence (SEQ ID NO:3).

```
Native AUGAUAAU---AUG aCu uCg Aaa gUu uAu gAu cca gaa caa  
Mutant AUGAUAAgcuugccacaacccgggauccucuagagucgacAUG acu ucg
```

Figure 12. Plasmid pSN01-M1.

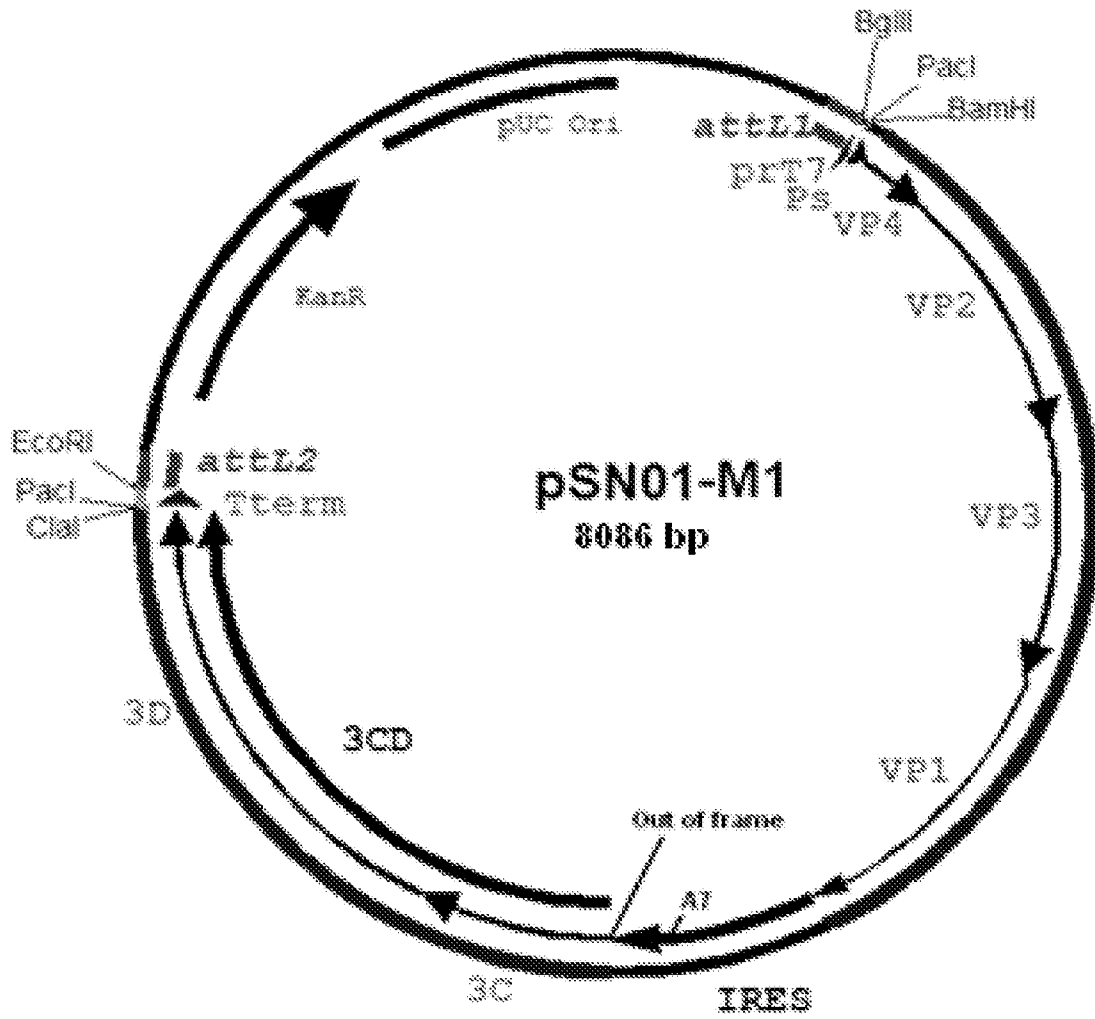


Figure 13. Plasmid pSN01-M2

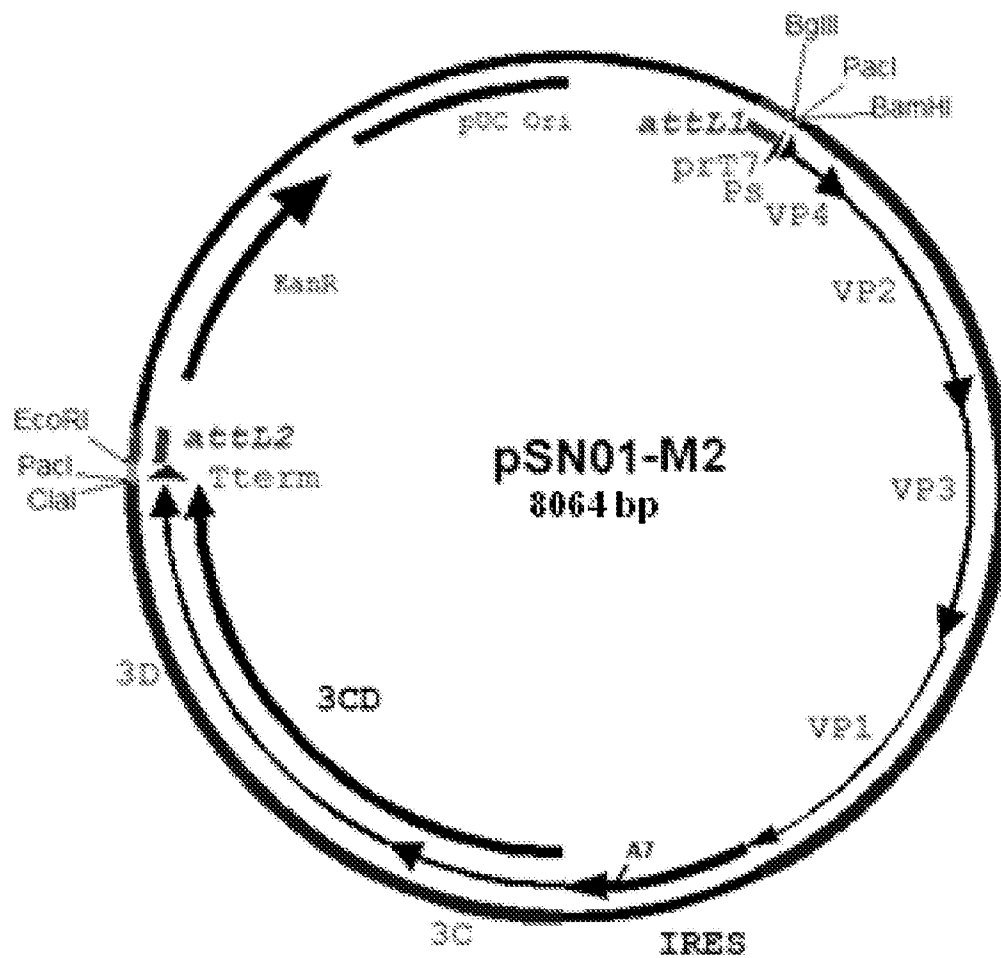


Figure 14. Plasmid pSN01-M3.

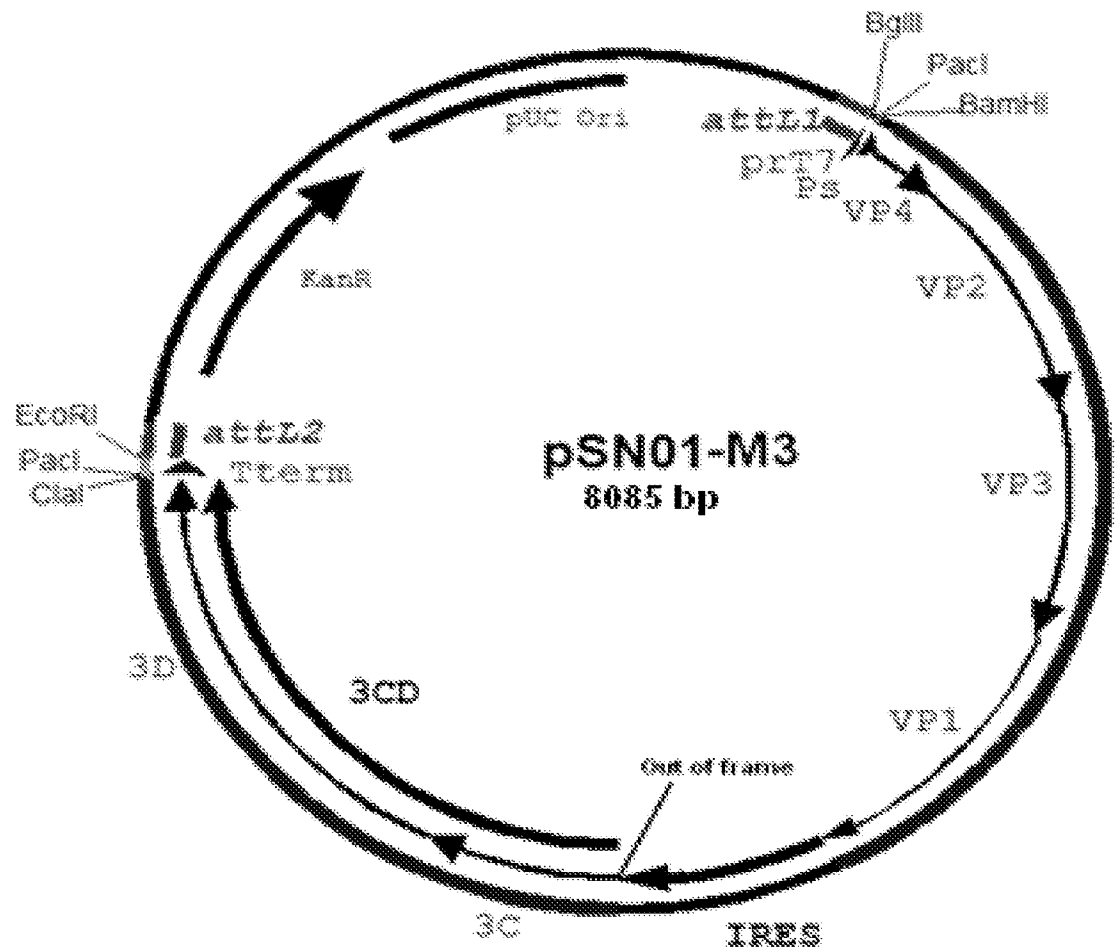


Figure 15. Comparison of expression of different recombinant baculovirus designs for expression of HEV71 VLP's.

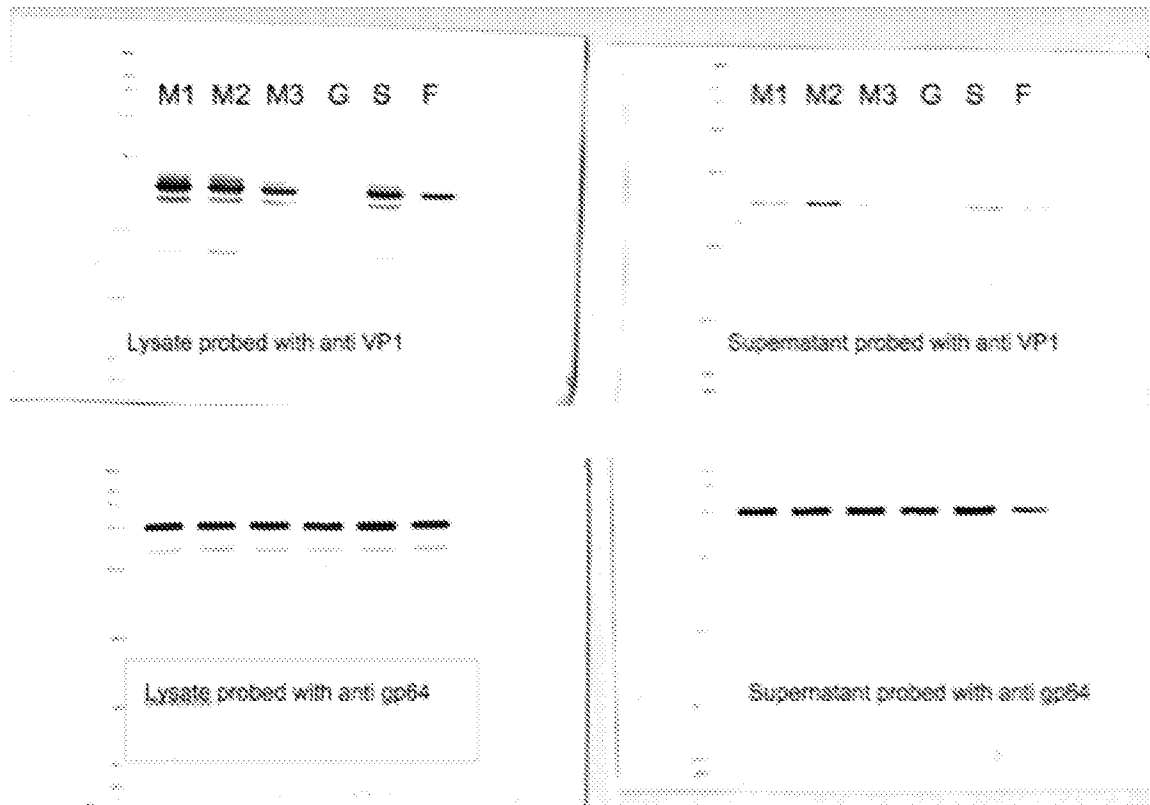


Figure 16. Plasmid pFastBac™ HT

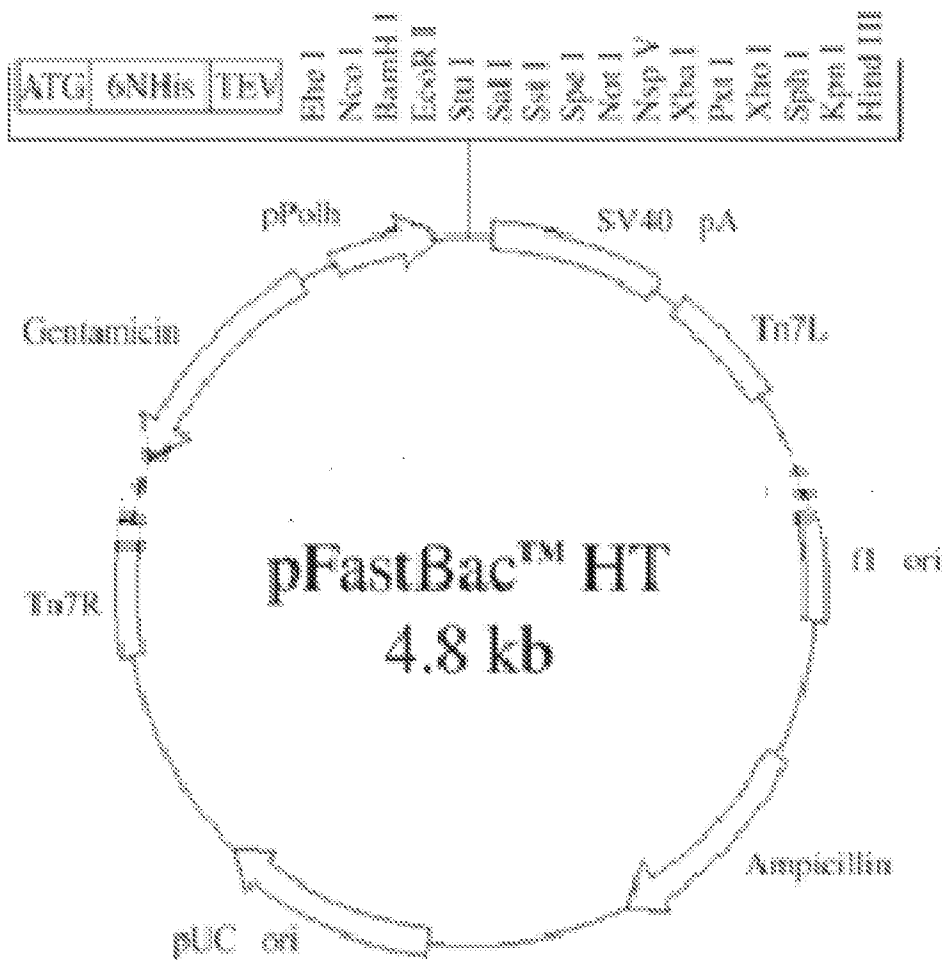


Figure 17. Prokaryotic expression construct for antigenic fusion proteins of *Human enterovirus A* and *Human enterovirus C*.

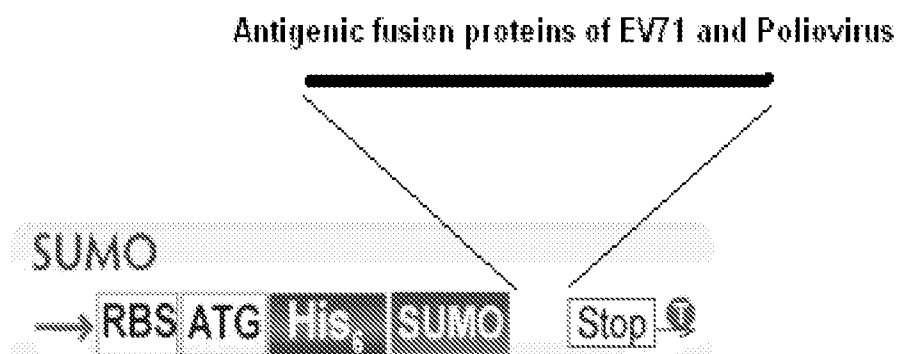


Figure 18. Antibodies from pooled neutralizing sera from mice immunized with SN07 retentate binds to all the components of HEV71 VLPs.

- 1. 1 ug of rEV71-VP0
- 2. 1 ug of rEV71-VP1
- 3. 1 ug of rEV71-VP2
- 4. 1 ug of rEV71-VP3

| kDa | 1 | 2 | 3 | 4 | kDa | 1 | 2 | 3 | 4 |
|-----|---|---|---|---|-----|---|---|---|---|
|-----|---|---|---|---|-----|---|---|---|---|

Figure 19. Characterization of HEV71 VLPs, pull-down of HEV71 VLPs from the culture supernatant. The VLP components (VP0, VP1 and VP3) are visible in the Coomassie stained SDS-PAGE gel. (MW- molecular weight marker; CB- Coomassie blue staining)

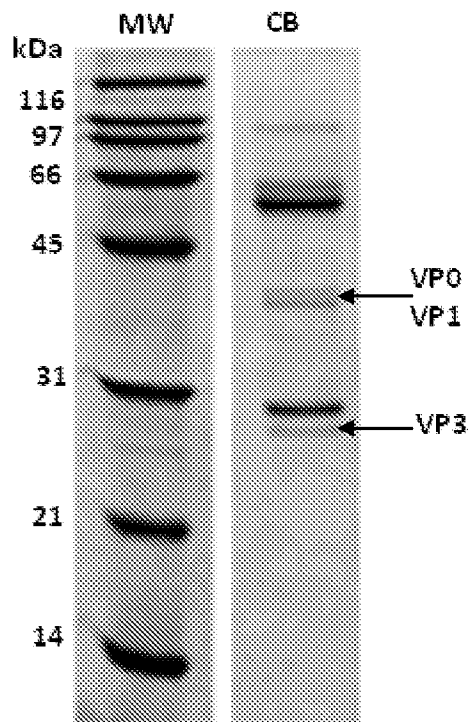


Figure 20. Analysis of affinity column (AFC) purified HEV71 VLPs.

AFC was prepared with a neutralizing monoclonal antibody (EV18/4/D6-1/F1/G9), which monoclonal antibody was developed using a SN07 retentate. The eluted fraction was analyzed by Western blotting. The eluted fraction in Lanes 1 and 2, probed using an anti-VP1 antibody, shows that VP1 is present in the AFC purified VLPs. The eluted fraction in Lanes 3 and 4, probed using an anti-VP2 antibody, shows that VP2 is also present in the AFC purified VLPs. The eluted fraction in Lanes 5 and 6, probed using an anti-VP2 monoclonal antibody, shows that VP2 is present in the AFC purified VLPs.

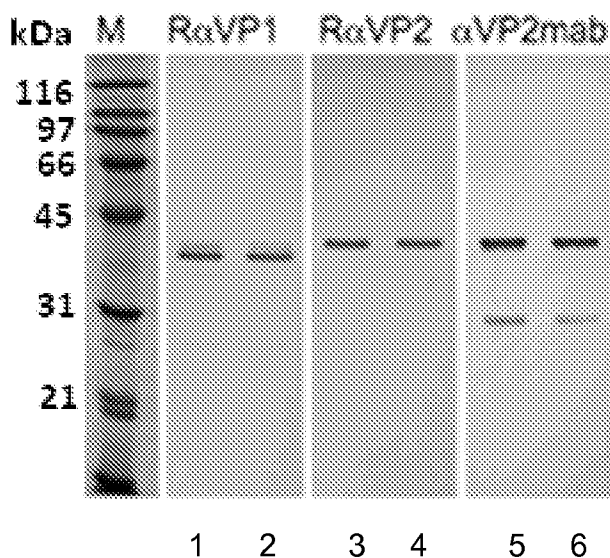


Figure 21. An electron micrograph picture of AFC purified HEV71 VLPs. AFC was prepared with a neutralizing monoclonal antibody (EV18/4/D6-1/F1/G9), which monoclonal antibody was developed using a SN07 retentate. The eluted fraction was analyzed by Western blotting (Figure 20) and electron microscopy. The arrows point to virus-like particles.

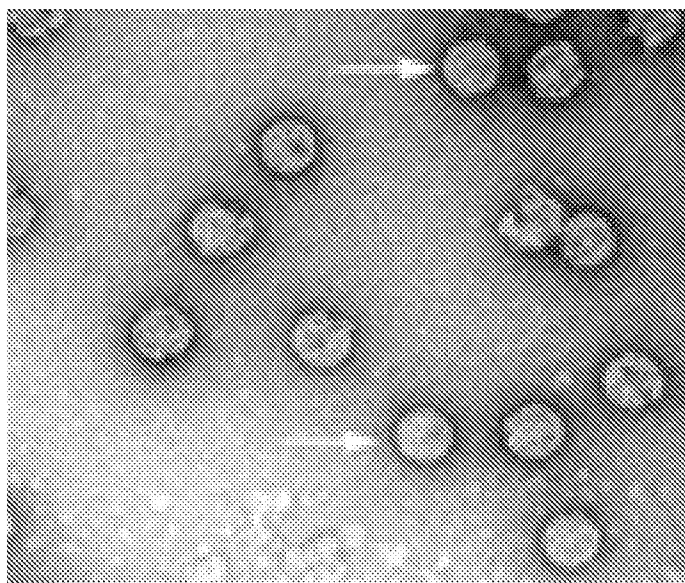


Figure 22. *Human enterovirus C* (poliovirus-PV) VL expression. Three different expression cassettes to generate poliovirus VLPs were constructed. The expression cassettes all comprise a poliovirus P1 polypeptide and differed with respect to the IRES, which IRES directs the expression of a poliovirus 3CD protease. Recombinant baculoviruses harboring the poliovirus VLP expression cassettes were tested. Lysates from the baculovirus infected cells were harvested on day 3 post infection and expression of the poliovirus VP3 was evaluated using rabbit anti-PVP3 antibodies (1:2000).

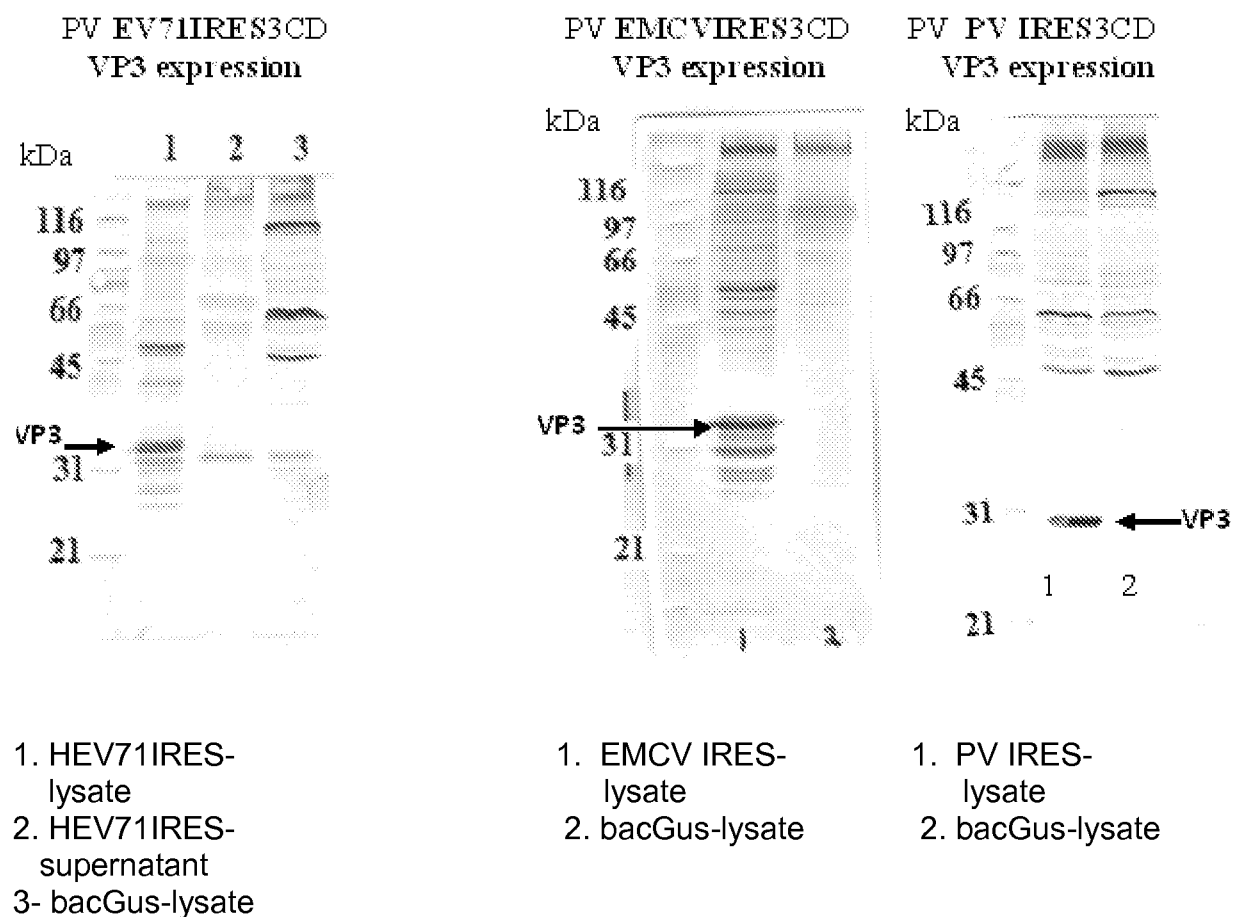


Figure 23. Poliovirus-VLP VP3-VP1 ELISA. To demonstrate poliovirus VLP generation, a two sites ELISA was performed using lysates and supernatants from recombinant baculoviruses carrying a poliovirus VLP expression cassette wherein the poliovirus 3CD protease is under the control of a poliovirus IRES. Purified rabbit anti-poliovirus VP3 antibodies were used as capture antibodies. Poliovirus VLPs were detected using mouse anti-VP1 monoclonal antibodies.

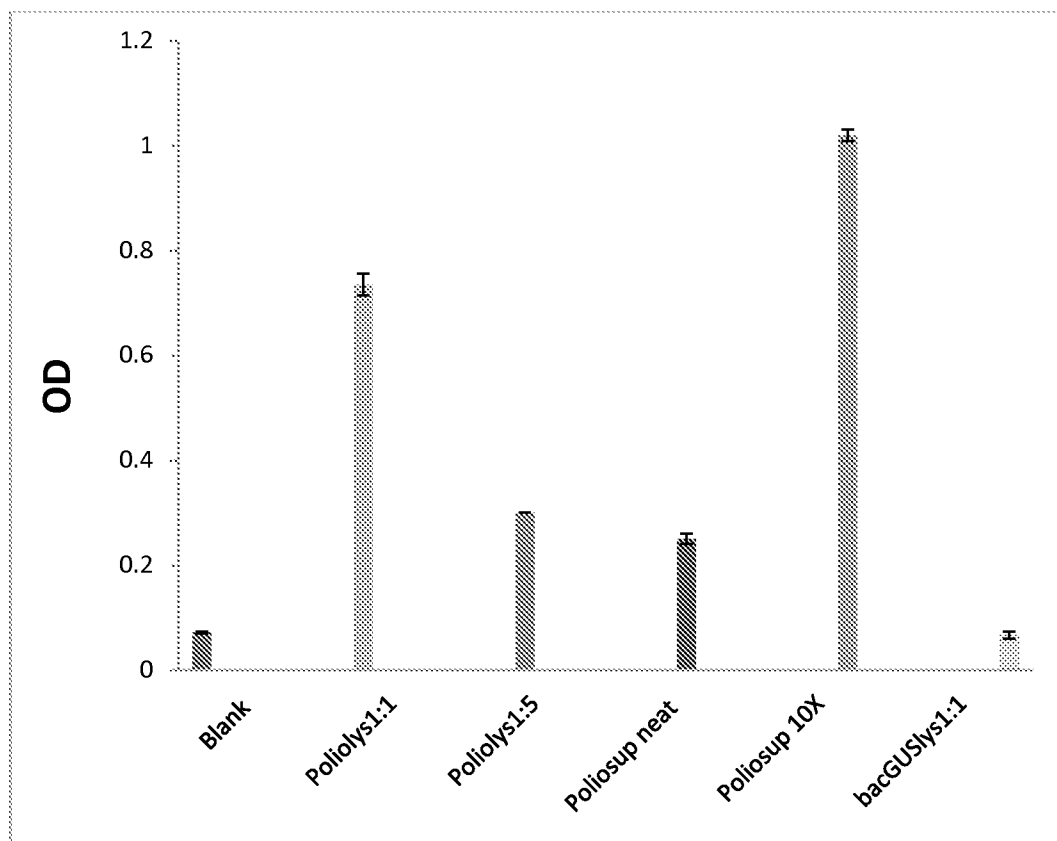


Figure 24. HEV71 VLP Expression Cassette with HEV71 IRES (P1+HEV71 IRES+3CD).

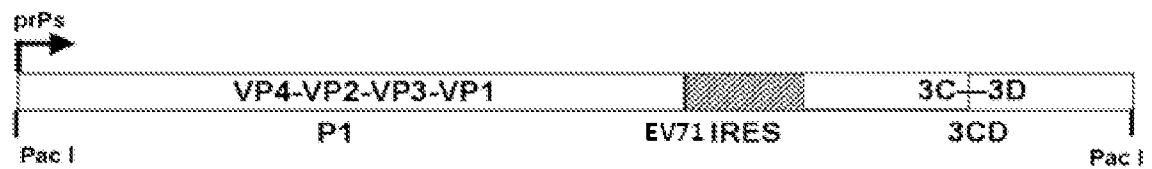


Figure 25. HEV71 VLP Expression Cassette with Poliovirus (PV) IRES (P1+PV IRES+3CD).

