Supplementary Materials

Supplementary material 1: Purified and crude rPA calculation for vaccine formulation

The vaccine formulation for the purified and crude rPA was calculated using:

$$C1V1 = C2V2.$$

C1 = Primary concentration

V1 = Primary volume

C2 = Final concentration

V2 = Final concentration

The concentration of the CrPA used for the vaccine formulation was determined with the 1 ml volume of the supernatant of the lysed cells after discarding the pellets following centrifugation. The formulas used to determine the rPA concentration in the crude whole supernatant are:

 $tx \ concn = wl \ concn - PrPA \ concn$ $ubrPA \ concn = tx \ concn - ft \ concn$ $CrPA \ concn = ubrPA + PrPA \ concn$

tx concn = Total protein concentration without the purified rPA concentration

wl concn = Whole protein concentration in supernatant after centrifugation

ubrPA concn = Concentration of unbind rPA concentration present in the flow through after passing the whole supernatant the Ni-TED column (Machery-Nagel, England).

ft concn = Concentration of the all proteins present in the flow through Ni-TED column.

CrPA concn = Concentration of rPA in the whole supernatant after centrifugation.

The CrPA vaccine was formulated as described for the PrPA vaccine formulation.