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

PrPA concn = Purified rPA concentration after purification using Ni-TED column (Machery-Nagel, England)

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.