

CLAIMS

1. A Zika virus vaccine comprising inactivated Zika virus particles, in which the Zika virus vaccine contains protamine sulphate (PS) or fragments thereof, in which PS or fragments of PS are present at amounts below 1 µg/mL and in which the vaccine is able to confer seroprotection on at least 70% of subjects that are administered the Zika virus vaccine.
2. A Zika virus vaccine according to Claim 1, in which the Zika virus vaccine is able to confer seroprotection on at least 80% of subjects that are administered the Zika virus vaccine.
3. A Zika virus vaccine according to Claim 1, in which the Zika virus vaccine is able to confer seroprotection on at least 90% of subjects that are administered the Zika virus vaccine.
4. A Zika virus vaccine according to Claim 1, in which the Zika virus vaccine is able to confer seroprotection on at least 95% of subjects that are administered the Zika virus vaccine.
5. A vaccine according to any preceding claim, in which the Zika virus particles comprise an RNA genome corresponding to the DNA sequence provided by any one of the nucleic acid sequences of SEQ ID NOs: 2-13 or 72, or a variant nucleic acid sequence that is at least 88% identical to any one of SEQ ID NOs: 2-13 or 72 and able to pack a virulent Zika virus.
6. A Zika virus vaccine according to any preceding claim, in which the Zika virus particles comprise an E protein selected from the amino acid sequences provided by any one of SEQ ID NOs: 14-69, or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 14-69 and able to pack a virulent Zika virus.
7. A Zika virus vaccine according to any preceding claim, in which the Zika virus particles are chemically inactivated, thermally inactivated, pH inactivated, or UV inactivated.
8. A Zika virus vaccine according to Claim 7, in which the chemical inactivation comprises contacting the Zika virus particles with a chemical inactivation agent for longer than is required to completely inactivate the Zika virus as measured by plaque assay.
9. A Zika virus vaccine according to Claim 7 or 8, in which the chemical inactivation comprises contacting the Zika virus particles with formaldehyde.

10. A Zika virus vaccine according to Claim 9, in which the formaldehyde inactivation comprises contacting the Zika virus particles with formaldehyde for between 2-10 days.
11. A Zika virus vaccine according to any of Claims 7 to 10, in which the chemical inactivation is performed at about +4°C or about +22°C.
- 5 12. A Zika virus vaccine according to any preceding claim, further comprising an adjuvant and/or one or more pharmaceutically acceptable excipients.
13. A Zika virus vaccine according to Claim 12, in which the adjuvant is an aluminium salt adjuvant.
14. A Zika virus vaccine according to Claim 13, in which the aluminium salt adjuvant is
10 aluminium hydroxide or aluminium phosphate salt.
15. The Zika virus vaccine according to Claim 14, in which the aluminium salt adjuvant is aluminium hydroxide with less than 1.25 ppb Cu based on the final pharmaceutical composition comprising the virus.
16. A Zika virus vaccine according to any of Claims 12 to 15, in which the Zika virus
15 vaccine comprises or further comprises an adjuvant comprising a peptide and a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN).
17. A Zika virus vaccine according to Claim 16, in which the peptide comprises the sequence KLKL5KLLK (SEQ ID NO: 71) and the I-ODN comprises oligo-d(IC)13 (SEQ ID NO: 70).
- 20 18. A Zika virus vaccine according to any preceding claim, in which the vaccine contains protamine sulphate or fragments thereof at amounts below 100 ng/mL.
19. A Zika virus vaccine according to Claim 18, in which the protamine sulphate or fragments thereof can be detected only by a highly sensitive method, in which the highly sensitive method is selected from mass spectroscopy and nuclear magnetic resonance.
- 25 20. A process for purification of inactivated Zika virus particles, comprising the steps of:

- (a) providing a crude harvest (a) comprising Zika virus particles and impurities, in which the impurities are generated from growing the Zika virus particles on a cell substrate;
- 5 (b) reducing impurities from crude harvest (a) by precipitation with an agent comprising a protamine salt, preferably a protamine sulphate, to obtain a Zika virus preparation (b);
- 10 (c) further purifying Zika virus preparation (b) by centrifugation on an optimized sucrose density gradient to obtain a Zika virus preparation (c) comprising the infectious Zika virus particles, in which the optimized sucrose density gradient is provided such that the protamine can be completely or almost completely separated from the virus fraction; and in which the protamine concentration is reduced by this step to the extent that the protamine concentration in the final drug substance is less than 1 µg/ml, preferably less than 900, 800, 700, 600, 500, 400, 300 or 200 ng/mL, preferably less than 100 ng/mL, as measured by a sensitive method selected from mass spectroscopy
- 15 and nuclear magnetic resonance, in which the optimized sucrose density gradient comprises a Zika virus comprising fraction in a 10% +/- 1% (w/w) sucrose solution and three layers of sucrose with different densities, i.e. a first sucrose solution with 15% +/- 1% (w/w) sucrose, a second sucrose solution with 35% +/- 1% (w/w) sucrose, and a third sucrose solution with a 50% +/- 1% (w/w) sucrose; and
- 20 (d) subjecting Zika virus preparation (c) to one or more inactivation steps.