

Claims

1. An aqueous immunogenic composition comprising
(i) a first RSV F protein trimer in the prefusion conformation;
5 (ii) sodium chloride at a concentration of between about 20 mM and about 250 mM;
(iii) at least one of sucrose, mannitol and glycine at a concentration of between about 5
mg/mL and about 100 mg/mL; and
(iv) a buffer;
wherein the pH of said composition is between about 7 and about 8.
- 10 2. The aqueous immunogenic composition according to claim 1, wherein sodium
chloride is at a concentration of between about 20 mM and about 100 mM.
- 15 3. The aqueous immunogenic composition according to claim 1 or 2, wherein sucrose is
at a concentration of between about 10 mg/mL and about 100 mg/mL.
- 20 4. The aqueous immunogenic composition according to any one of claims 1 to 3, wherein
glycine is at a concentration of between about 10 mg/mL and about 100 mg/mL or mannitol is at
a concentration of between about 10 mg/mL and about 100 mg/mL.
- 25 5. The aqueous immunogenic composition according to claim 1 or 2, wherein sucrose is
at a concentration of between about 10 mg/mL and about 70 mg/mL and mannitol is at a
concentration of between about 10 mg/mL and about 70 mg/mL.
- 30 6. The aqueous immunogenic composition according to claim 5, wherein the
concentration ratio of sucrose to mannitol is between 1 to 1 and 1 to 5, preferably between 1 to
2 and 1 to 4.
7. The aqueous immunogenic composition according to any one of claims 1 to 6, wherein
the composition further comprises a surfactant.
8. The aqueous immunogenic composition according to claim 7, wherein the surfactant
is polysorbate 80.
- 35 9. The aqueous immunogenic composition according to any one of claims 1 to 8, wherein
the buffer is Tris (tris(hydroxymethyl) aminomethane).

10. The aqueous immunogenic composition according to claim 1, wherein the composition comprises

- (i) a first RSV F protein trimer in the prefusion conformation;
 - (ii) sodium chloride at a concentration of between about 35 mM and about 65 mM;
 - 5 (iii) sucrose at a concentration of between about 20 mg/mL and about 40 mg/mL and mannitol at a concentration of between about 45 mg/mL and about 75 mg/mL;
 - (iv) a Tris buffer at a concentration of between about 15 mM and about 25 mM;
 - (v) polysorbate 80 at a concentration of between about 0.1 and about 0.3 mg/mL;
- wherein the pH of the composition is between 7.1 and 7.7.

11. The aqueous immunogenic composition according to claim 1, wherein the composition comprises

- (i) a first RSV F protein trimer in the prefusion conformation;
 - (ii) sodium chloride at a concentration of about 50 mM;
 - 15 (iii) sucrose at a concentration of about 30mg/mL and mannitol at a concentration of about 60 mg/mL;
 - (iv) a Tris buffer at a concentration of about 20mM;
 - (v) polysorbate 80 at a concentration of about 0.2 mg/mL;
- wherein the pH of the composition is about 7.4.

12. The aqueous immunogenic composition according to any one of claims 1 to 11, wherein the first RSV F protein is a F protein of subtype A.

13. The aqueous immunogenic composition according to claim 12, wherein the first RSV F protein comprises a combination of mutations relative to the corresponding wild-type RSV F protein, wherein the combination of mutations is selected from the group consisting of:

- (1) combination of 103C, 148C, 190I, and 486S, preferably A103C, I148C, S190I, and D486S;
- (2) combination of 54H, 55C, 188C, and 486S, preferably T54H, S55C, L188C, and D486S;
- 30 (3) combination of 54H, 103C, 148C, 190I, 296I, and 486S, preferably T54H, A103C, I148C, S190I, V296I, and D486S;
- (4) combination of 54H, 55C, 142C, 188C, 296I, and 371C, preferably T54H, S55C, L142C, L188C, V296I, and N371C;
- (5) combination of 55C, 188C, and 486S, preferably S55C, L188C, and D486S;
- 35 (6) combination of 54H, 55C, 188C, and 190I, preferably T54H, S55C, L188C, and S190I;
- (7) combination of 55C, 188C, 190I, and 486S, preferably S55C, L188C, S190I, and D486S;
- (8) combination of 54H, 55C, 188C, 190I, and 486S, preferably T54H, S55C, L188C, S190I, and D486S;

(9) combination of 155C, 190I, 290C, and 486S, preferably S155C, S190I, S290C, and D486S;

(10) combination of 54H, 55C, 142C, 188C, 296I, 371C, 486S, 487Q, and 489S, preferably T54H, S55C, L142C, L188C, V296I, N371C, D486S, E487Q, and D489S;

5 (11) combination of 54H, 155C, 190I, 290C, and 296I, preferably T54H, S155C, S190I, S290C, and V296I,

(12) combination of 155C, 190F, 290C, and 207L, preferably S155C, S190F, S290C, and V207L,

(13) combination of 215P and 486N, preferably S215P and D486N,

10 (14) combination of 66E, 215P, and 486N, preferably K66E, S215P, and D486N,

(15) combination of 66E, 76V, 215P, and 486N, preferably K66E, I76V, S215P, and D486N, and,

(16) combination of 66E, 67I, 76V, 215P, and 486N, preferably K66E, N67I, I76V, S215P, and D486N.

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14. The aqueous immunogenic composition according to claim 12 or 13, wherein the first RSV F protein comprises a trimerization domain.

15. The aqueous immunogenic composition according to any one of claims 1 to 14, wherein the composition further comprises a second RSV F protein trimer in the prefusion conformation.

16. The aqueous immunogenic composition according to claim 15, wherein the second RSV F protein is a F protein of subtype B.

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17. The aqueous immunogenic composition according to claim 15 or 16, wherein the second RSV F protein comprises a combination of mutations relative to the corresponding wild-type RSV F protein, wherein the combination of mutations is selected from the group consisting of:

30 (1) combination of 103C, 148C, 190I, and 486S, preferably A103C, I148C, S190I, and D486S;

(2) combination of 54H, 55C, 188C, 486S, preferably T54H, S55C, L188C, and D486S;

(3) combination of 54H, 103C, 148C, 190I, 296I, and 486S, preferably T54H, A103C, I148C, S190I, V296I, and D486S;

35 (4) combination of 54H, 55C, 142C, 188C, 296I, and 371C, preferably T54H, S55C, L142C, L188C, V296I, and N371C;

(5) combination of 55C, 188C, and 486S, preferably S55C, L188C, and D486S;

(6) combination of 54H, 55C, 188C, and 190I, preferably T54H, S55C, L188C, and S190I;

(7) combination of 55C, 188C, 190I, and 486S, preferably S55C, L188C, S190I, and D486S;
(8) combination of 54H, 55C, 188C, 190I, and 486S, preferably T54H, S55C, L188C, S190I, and D486S;

5 (9) combination of 155C, 190I, 290C, and 486S, preferably S155C, S190I, S290C, and D486S;

(10) combination of 54H, 55C, 142C, 188C, 296I, 371C, 486S, 487Q, and 489S, preferably T54H, S55C, L142C, L188C, V296I, N371C, D486S, E487Q, and D489S;

(11) combination of 54H, 155C, 190I, 290C, and 296I, preferably T54H, S155C, S190I, S290C, and V296I,

10 (12) combination of 155C, 190F, 290C, and 207L, preferably S155C, S190F, S290C, and V207L,

(13) combination of 215P and 486N, preferably S215P and D486N,

(14) combination of 66E, 215P, and 486N, preferably K66E, S215P, and D486N,

15 (15) combination of 66E, 76V, 215P, and 486N, preferably K66E, I76V, S215P, and D486N, and,

(16) combination of 66E, 67I, 76V, 215P, and 486N, preferably K66E, N67I, I76V, S215P, and D486N.

20 18. The aqueous immunogenic composition according to any one of claims 15 to 17, wherein the second RSV F protein comprises a trimerization domain.

25 19. A method for storing an aqueous immunogenic composition comprising an RSV F protein trimer in the prefusion conformation comprising storing the composition at a temperature of at least about 15 °C.

20. The method according to claim 19, wherein the temperature is of between about 15°C and about 30 °C.

30 21. The method according to claim 19 or 20, wherein the RSV F protein is a F protein of subtype A or a F protein of subtype B.

22. The method according to claim 19 or 20, wherein the aqueous immunogenic composition is a composition according to any one of claims 1 to 18.

35 23. A method for lyophilizing an aqueous immunogenic composition comprising an RSV F protein trimer in the prefusion conformation comprising the step of freezing the composition wherein said step comprises reducing the temperature to a freezing temperature comprised between -40 °C and -60 °C and at a freezing ramp rate of at least about 0.3 °C/min.

24. A method for lyophilizing an aqueous immunogenic composition comprising an RSV F protein trimer in the prefusion conformation comprising the following steps:

(a) providing an aqueous composition comprising an RSV F protein trimer in the prefusion conformation,

(b) freezing the composition wherein said step comprises reducing the temperature to a freezing temperature comprised between about -40 °C and about -60 °C and at a freezing ramp rate of at least about 0.3 °C/min (for example between about 0.3 and about 2 °C/min), and

maintaining the composition at the freezing temperature for at least about 30 min, for example between 30 mins and 120 mins;

(c) annealing the frozen composition;

(d) refreezing the annealed composition wherein said step comprises reducing the temperature to a refreezing temperature comprised between about -40 °C and about -60 °C and at a refreezing ramp rate of at least 0.3 °C/min, and

maintaining the composition at the refreezing temperature for at least about 30 min, for example between 30 mins and 120 mins;

(e) drying the refrozen composition, and

(f) obtaining a lyophilized composition comprising the RSV F protein trimer in the prefusion conformation.

25. A lyophilized immunogenic composition obtained or obtainable by the method according to claim 23 or 24.

26. A kit comprising:

- (i) a lyophilized composition according to claim 25, and
- (ii) a diluent for reconstituting the lyophilized composition.

27. A kit comprising:

- (i) a lyophilized composition, and
- (ii) a diluent for reconstituting the lyophilized composition;

wherein the reconstitution of the lyophilized composition with the diluent results in an aqueous immunogenic composition according to any one of claims 1 to 18.

28. A kit according to claim 26 or 27, wherein the diluent is water for injection.

29. A kit according to any one of claims 26 to 28, wherein the diluent comprises a preservative.

30. A kit according to claim 29, wherein the preservative is 2-phenoxyethanol.

31. A kit comprising:

(i) a lyophilized composition, and

5 (ii) a diluent comprising sodium chloride at a concentration of between about 20 mM and about 300 mM;

wherein the reconstitution of the lyophilized composition with the diluent results in an aqueous immunogenic composition according to any one of claims 1 to 18.

10 32. A kit according to claim 31, wherein the diluent comprises a preservative.

33. A kit according to claim 32, wherein the preservative is 2-phenoxyethanol.