CLAIMS

1. A method for providing single-stranded RNA (ssRNA), comprising:
   (i) providing an RNA preparation comprising ssRNA produced by *in vitro* transcription;
   (ii) contacting the RNA preparation with a cellulose material under conditions which allow binding of double-stranded RNA (dsRNA) to the cellulose material; and
   (iii) separating the ssRNA from the cellulose material under conditions which allow binding of dsRNA to the cellulose material.

2. The method of claim 1, further comprising the step of producing the RNA preparation comprising ssRNA by *in vitro* transcription.

3. The method of claim 1 or 2, wherein steps (ii) and (iii) are conducted under conditions which allow binding of dsRNA to the cellulose material and do not allow binding of ssRNA to the cellulose material.

4. The method of claim 3, wherein step (ii) comprises mixing the RNA preparation comprising ssRNA with the cellulose material under shaking and/or stirring, preferably for at least 5 min, more preferably for at least 10 min.

5. The method of claim 4, wherein in step (ii) the RNA preparation is provided as a liquid comprising ssRNA and a first buffer and/or the cellulose material is provided as a suspension in a first buffer, wherein the first buffer comprises water, ethanol and a salt, preferably sodium chloride, in a concentration which allows binding of dsRNA to the cellulose material and which does not allow binding of ssRNA to the cellulose material.

6. The method of claim 5, wherein the concentration of ethanol in the first buffer is 14 to 20% (v/v), preferably 14 to 16% (v/v).

7. The method of claim 5 or 6, wherein the concentration of the salt in the first buffer is 15 to 70 mM, preferably 20 to 60 mM.

8. The method of any one of claims 5 to 7, wherein the first buffer further comprises a buffering substance, preferably tris(hydroxymethyl)aminomethane (TRIS), and/or a chelating agent, preferably EDTA.

9. The method of any one of claims 5 to 8, wherein in step (iii) the mixture of the RNA preparation, the cellulose material, and the first buffer is provided in a tube and step (iii) comprises (1) applying gravity or centrifugal force to the tube such that the liquid and solid phases are separated; and (2) either collecting the supernatant comprising ssRNA or removing the cellulose material.

10. The method of any one of claims 5 to 8, wherein in step (iii) the mixture of the RNA preparation, the cellulose material, and the first buffer is provided in a spin column or filter device and step (iii) comprises (1') applying gravity, centrifugal force, pressure, or vacuum to the spin column or filter device such that the liquid and solid phases are separated; and (2') collecting the flow through comprising ssRNA.

11. The method of any one of claims 3 to 10, wherein steps (ii) and (iii) are repeated once or two or more times, wherein the ssRNA preparation obtained after step (iii) of one cycle of steps (ii) and (iii) is used as RNA preparation in step (ii) of the next cycle and in step (ii) of each cycle of steps (ii) and (iii) fresh cellulose material is used.

12. The method of claim 1 or 2, wherein step (ii) is conducted under conditions which allow binding of dsRNA and ssRNA to the cellulose material; and step (iii) is conducted under conditions which allow binding of dsRNA to the cellulose material and do not allow binding of ssRNA to the cellulose material.

13. The method of claim 12, wherein step (ii) comprises (1) mixing the RNA preparation comprising ssRNA with the cellulose material under shaking and/or stirring, preferably for at least 5 min, more preferably for at least 10 min; and (2) separating the cellulose material to which dsRNA and ssRNA are bound from the remainder.

14. The method of claim 13, wherein in step (ii) the RNA preparation is provided as a liquid comprising ssRNA and a second buffer and/or the cellulose material is provided as a suspension in a second buffer, wherein the second buffer comprises water, ethanol and a salt, preferably sodium chloride, in a concentration which allows binding of dsRNA and ssRNA to the cellulose material.

15. The method of claim 14, wherein the concentration of ethanol in the second buffer is at least 35% (v/v), preferably 38 to 42% (v/v).

16. The method of claim 14 or 15, wherein the concentration of the salt in the second buffer is 15 to 70 mM, preferably 20 to 60 mM.

17. The method of any one of claims 14 to 16, wherein the second buffer further comprises a buffering substance, preferably tris(hydroxymethyl)aminomethane (TRIS), and/or a chelating agent, preferably EDTA.

18. The method of any one of claims 14 to 17, wherein in step (ii)(2) the mixture of the RNA preparation and the cellulose material obtained in step (ii)(1) is provided in a tube and step (ii)(2) comprises (2a) applying gravity or centrifugal force to the tube such that the liquid and solid phases are separated; and (2b) either removing the supernatant or collecting the cellulose material to which dsRNA and ssRNA are bound.

19. The method of any one of claims 14 to 17, wherein in step (ii)(2) the mixture of the RNA preparation and the cellulose material obtained in step (ii)(1) is provided in a spin column or filter device and step (ii)(2) comprises (2a') applying gravity, centrifugal force, pressure, or vacuum to the spin column or filter device such that the liquid and solid phases are separated; and (2b') discarding the flow through.

20. The method of any one of claims 14 to 19, wherein step (ii) further comprises (3) adding an aliquot of the second buffer to the cellulose material to which dsRNA and ssRNA are bound; (4) incubating the resulting mixture under shaking and/or stirring, preferably for at least 5 min, more preferably for at least 10 min; and (5) separating the cellulose material to which dsRNA and ssRNA are bound from the liquid phase; and optionally (6) repeating steps (3) to (5) once or two or more times.

21. The method of any one of claims 12 to 20, wherein step (iii) comprises (1) mixing the cellulose material to which dsRNA and ssRNA are bound with a first buffer under shaking and/or stirring, preferably for at least 5 min, more preferably for at least 10 min, wherein the first buffer comprises water, ethanol and a salt, preferably sodium chloride, in a concentration which allows binding of dsRNA to the cellulose material and does not allow binding of ssRNA to the cellulose material; and (2) separating the liquid phase comprising ssRNA from the cellulose material.

22. The method of claim 21, wherein the concentration of ethanol in the first buffer is 14 to 20% (v/v), preferably 14 to 16% (v/v).

23. The method of claim 21 or 22, wherein the concentration of the salt in the first buffer is 15 to 70 mM, preferably 20 to 60 mM.

24. The method of any one of claims 21 to 23, wherein the first buffer further comprises a buffering substance, preferably tris(hydroxymethyl)aminomethane (TRIS), and/or a chelating agent, preferably EDTA.

25. The method of any one of claims 21 to 24, wherein in step (iii) the mixture of the cellulose material and the first buffer is provided in a tube and step (iii)(2) comprises (2a) applying gravity or centrifugal force to the tube such that the liquid and solid phases are separated; and (2b) either collecting the supernatant comprising ssRNA or removing the cellulose material.

26. The method of any one of claims 21 to 24, wherein in step (iii) the mixture of the cellulose material and the first buffer is provided in a spin column or filter device and step (iii)(2) comprises (2a') applying gravity, centrifugal force, pressure, or vacuum to the spin column or filter device; and (2b') collecting the flow through comprising ssRNA.

27. The method of any one of claims 12 to 26, wherein steps (ii) and (iii) are repeated once or two or more times, wherein the ssRNA preparation obtained after step (iii) of one cycle of steps (ii) and (iii) is used as RNA preparation in step (ii) of the next cycle and in step (ii) of each cycle of steps (ii) and (iii) fresh cellulose material is used.

28. The method of claim 12, wherein in step (ii) the cellulose material is provided in a column, step (ii) comprises loading the RNA preparation onto the column under conditions which allow binding of dsRNA and ssRNA to the cellulose material, and step (iii) comprises eluting the ssRNA from the cellulose material under conditions which allow binding of dsRNA to the cellulose material and do not allow binding of ssRNA to the cellulose material.

29. The method of claim 28, wherein in step (ii) the RNA preparation is provided and loaded on the column as a liquid comprising ssRNA and a second buffer, wherein the second buffer comprises water, ethanol and a salt, preferably sodium chloride, in a concentration which allows binding of dsRNA and ssRNA to the cellulose material.

30. The method of claim 29, wherein the concentration of ethanol in the second buffer is at least 35% (v/v), preferably 38 to 42% (v/v).

31. The method of claim 29 or 30, wherein the concentration of the salt in the second buffer is 15 to 70 mM, preferably 20 to 60 mM.

32. The method of any one of claims 29 to 31, wherein the second buffer further comprises a buffering substance, preferably tris(hydroxymethyl)aminomethane (TRIS), and/or a chelating agent, preferably EDTA.

33. The method of any one of claims 28 to 32, wherein step (iii) is conducted using a first buffer as eluent, wherein the first buffer comprises water, ethanol and a salt, preferably sodium chloride, in a concentration which allows binding of dsRNA to the cellulose material and does not allow binding of ssRNA to the cellulose material.

34. The method of claim 33, wherein the concentration of ethanol in the first buffer is 14 to 20% (v/v), preferably 14 to 16% (v/v).

35. The method of claim 33 or 34, wherein the concentration of the salt in the first buffer is 15 to 70 mM, preferably 20 to 60 mM.

36. The method of any one of claims 33 to 35, wherein the first buffer further comprises a buffering substance, preferably tris(hydroxymethyl)aminomethane (TRIS), and/or a chelating agent, preferably EDTA.

37. The method of any one of claims 1 to 36, wherein the RNA preparation is produced by using an RNA polymerase selected from the group consisting of T3, T7 and SP6 RNA polymerases.

38. The method of any one of claims 1 to 37, wherein prior to step (ii) the RNA preparation is subjected to at least one pre-purification treatment.

39. The method of claim 38, wherein the at least one pre-purification treatment comprises one or more of the following: precipitation of nucleic acids, preferably using lithium chloride; binding of nucleic acids to magnetic beads; ultrafiltration; and degradation of DNA, preferably using duplex-specific nuclease (DSN).

40. The method of any one of claims 1 to 39, wherein the ssRNA is mRNA or an inhibitory RNA, such as antisense RNA, siRNA, or miRNA.

41. The method of any one of claims 1 to 40, wherein the ssRNA has a length of at least 2700 nt, preferably at least 3000 nt, more preferably at least 3500 nt, more preferably at least 4500 nt.

42. The method of any one of claims 1 to 41, wherein the cellulose material comprises cellulose fibers, preferably cellulose fibers of a grade suitable for use as a partition chromatography reagent.

43. The method of any one of claims 1 to 42, wherein prior to contacting with the RNA preparation in step (ii) the cellulose material is provided as a washed cellulose material.

44. The method of claim 43, wherein the washing of the cellulose material includes (I) mixing the cellulose material with a washing solution under shaking and/or stirring, preferably for at least 5 min, more preferably for at least 10 min; and (II) either removing the liquid or collecting the cellulose material; and optionally (III) repeating steps (I) and (II) once or two or more times.

45. The method of claim 44, wherein the washing solution has the composition of (A) the first buffer defined in any one of claims 5 to 8 if step (ii) is conducted under conditions which allow binding of dsRNA to the washed cellulose material and do not allow binding of ssRNA to the washed cellulose material, or (B) the second buffer defined in any one of claims 14 to 17 if step (ii) is conducted under conditions which allow binding of dsRNA and ssRNA to the washed cellulose material.

46. ssRNA obtainable by the method of any one of claims 1 to 45.

47. The ssRNA of claim 46, which is substantially free of dsRNA, preferably substantially free of dsRNA and DNA.

48. The ssRNA of claim 46 or 47 for use in therapy.

CLAIMS

1. A method for providing single-stranded RNA (ssRNA), comprising:

(i) providing an RNA preparation, wherein the RNA preparation comprises ssRNA and has been or is produced by *in vitro* transcription;

(ii) contacting the RNA preparation with a cellulose material under conditions which allow binding of double-stranded RNA (dsRNA) to the cellulose material and do not allow binding of the ssRNA to the cellulose material; and

(iii) separating the ssRNA from the cellulose material under conditions which allow binding of the dsRNA to the cellulose material and do not allow binding of the ssRNA to the cellulose material,

wherein in step (ii) the RNA preparation is provided as a liquid comprising the ssRNA and a first buffer and/or the cellulose material is provided as a suspension in the first buffer, wherein the first buffer comprises water, ethanol and a salt in a concentration which allows binding of the dsRNA to the cellulose material and which does not allow binding of the ssRNA to the cellulose material, and the concentration of ethanol in the first buffer is 14 to 20% (v/v).

2. The method of claim 1, wherein step (ii) comprises mixing the RNA preparation comprising ssRNA with the cellulose material under shaking and/or stirring; or
wherein step (ii) comprises mixing the RNA preparation comprising ssRNA with the cellulose material under shaking and/or stirring for at least 5 min.

3. The method of claim 1 or 2, wherein in step (iii) the mixture of the RNA preparation, the cellulose material, and the first buffer is provided in a tube and step (iii) comprises (1) applying gravity or centrifugal force to the tube such that the liquid and solid phases are separated; and (2) either collecting the supernatant comprising ssRNA or removing the cellulose material; or
wherein in step (iii) the mixture of the RNA preparation, the cellulose material, and the first buffer is provided in a spin column or filter device and step (iii) comprises (1') applying gravity, centrifugal force, pressure, or vacuum to the spin column or filter device such that the liquid and solid phases are separated; and (2') collecting the flow through comprising ssRNA.

4. The method of any one of claims 1 to 3, wherein steps (ii) and (iii) are repeated once or two or more times, wherein the ssRNA preparation obtained after step (iii) of one cycle of steps (ii) and (iii) is used as RNA preparation in step (ii) of the next cycle and in step (ii) of each cycle of steps (ii) and (iii) fresh cellulose material is used.

5. The method of any one of claims 1 to 4, wherein the first buffer comprises a buffering substance in a concentration of 5 to 40 mM.

6. The method of claim 5, wherein the buffering substance is tris(hydroxymethyl)aminomethane (TRIS).

7. The method of any one of claims 1 to 6, wherein the concentration of the salt in the first buffer is 15 to 70 mM or 20 to 60 mM.

8. The method of any one of claims 1 to 7, wherein the first buffer comprises a chelating agent, or wherein the first buffer comprises a chelating agent in a concentration of 10 to 50 mM.

9. The method of any one of claims 1 to 8, wherein the first buffer comprises:

(i) 14 to 20% (v/v) ethanol;
5 to 40 mM TRIS; and
15 to 70 mM salt; or

(ii) 14 to 20% (v/v) ethanol;
5 to 40 mM TRIS;
15 to 70 mM salt; and
10 to 50 mM chelating agent.

10. The method of claim 8 or 9, wherein the chelating agent is EDTA.

11. The method of claim 5, wherein the buffering substance is 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES).

12. The method of claim 11, wherein the concentration of the salt in the first buffer is 100 to 150 mM or 110 to 140 mM.

13. The method of claim 11 or 12, wherein the first buffer comprises:
14 to 20% (v/v) ethanol;
5 to 40 mM HEPES; and
110 to 140 mM salt.

14. The method of any one of claims 1 to 13, wherein the concentration of ethanol in the first buffer is 14 to 16% (v/v), and/or the salt in the first buffer is sodium chloride.

15. The method of any one of claims 1 to 14, wherein

(i) the RNA is produced or has been produced by using a DNA-dependent RNA polymerase,

(ii) the RNA is produced or has been produced by using a DNA-dependent RNA polymerase selected from the group consisting of T3, T7 and SP6 RNA polymerases,

(iii) prior to step (ii) the RNA preparation is subjected to at least one pre-purification treatment,

(iv) prior to step (ii) the RNA preparation is subjected to at least one pre-purification treatment, wherein the at least one pre-purification treatment comprises one or more of the following: precipitation of nucleic acids; binding of nucleic acids to magnetic beads; ultrafiltration; and degradation of DNA, and/or

(v) prior to step (ii) the RNA preparation is subjected to at least one pre-purification treatment, wherein the at least one pre-purification treatment comprises precipitation of nucleic acids which is performed by using lithium chloride, or wherein the at least one pre-purification treatment comprises degradation of DNA which is performed by using duplex-specific nuclease (DSN).

16. The method of any one of claims 1 to 15, wherein

(i) the ssRNA is mRNA, an inhibitory RNA, antisense RNA, siRNA, or miRNA, and/or

(ii) the ssRNA has a length of at least 2700 nt.

17. The method of any one of claims 1 to 16, wherein

(i) the cellulose material comprises cellulose fibers of a grade suitable for use as a partition chromatography reagent,

(ii) prior to contacting with the RNA preparation in step (ii) the cellulose material is provided as a washed cellulose material, and/or

(iii) prior to contacting with the RNA preparation in step (ii) the cellulose material is provided as a washed cellulose material, wherein the washing of the cellulose material includes (I) mixing the cellulose material with a washing solution under shaking and/or stirring; and (II) either removing the liquid or collecting the cellulose material; and optionally (III) repeating steps (I) and (II) once or two or more times, wherein, preferably, the washing solution has the composition of the first buffer defined in any one of claims 1 and 5 to 14.

18. ssRNA obtained by the method of any one of claims 1 to 17.

19. The ssRNA of claim 18, which is substantially free of dsRNA, preferably substantially free of dsRNA and DNA.

20. The ssRNA of claim 18 or claim 19 for use in therapy.