

## Claims

1. A method of isolating a target cell, wherein the target cell has a receptor molecule on the target cell surface, the method comprising:

providing a sample comprising one or more target cells;

providing a receptor binding reagent that is a monovalent antibody fragment comprising a monovalent binding site B and a binding partner C, wherein:

the monovalent binding site B is capable of specifically binding to the receptor molecule on the target cell surface with a dissociation constant ( $K_D$ ) that is of low affinity, wherein said low affinity is in the range of about  $10^{-3}$  to about  $10^{-7}$  M, or with a dissociation rate constant ( $k_{off}$ ) of about  $3 \times 10^{-5} \text{ sec}^{-1}$  or greater; and

the binding partner C comprises a streptavidin binding peptide,

exposing the sample to chromatography on a stationary phase, the stationary phase having an affinity reagent comprising a streptavidin mutein immobilized thereon, wherein the affinity reagent comprises a binding site Z capable of forming a non-covalent reversible complex with the binding partner C, and wherein the monovalent binding site B is capable of binding to the receptor molecule on the target cell surface, thereby reversibly immobilizing the one or more target cells on the stationary phase; and

eluting the one or more target cells from the chromatography matrix, thereby isolating at least one target cell that is free from a bound receptor binding reagent.

2. The method of claim 1, wherein the affinity reagent comprises two or more binding sites Z capable of reversibly binding to the binding partner C comprised in the receptor binding reagent.

3. The method of claim 1 or claim 2, wherein the sample comprises a mixture of target cells and further cells, wherein the further cells lack said receptor molecule on their cell surface, and the method further comprises separating the target cell from the further cells.

4. The method of any of claims 1-3, wherein prior to eluting, further comprising loading onto the stationary phase a competition reagent, wherein the competition reagent is capable of competitively binding to said binding site Z of the affinity reagent to disrupt the

binding of the binding partner C of the receptor binding reagent to the binding site Z of the affinity reagent, thereby displacing the receptor binding reagent from the affinity reagent.

5. The method of claim 4, comprising collecting an eluate from the stationary phase comprising at least one target cell.

6. The method of any of claims 1 to 5, further comprising forming said sample, wherein forming the sample comprises:

providing a source sample, the source sample comprising the one or more target cells;  
and

adding the receptor binding reagent to the source sample.

7. The method of claim 6, wherein an excess of receptor binding reagent is provided relative to the number of the one or more target cells.

8. The method of any of claims 1-5, wherein the receptor binding reagent is immobilised on the stationary phase prior to applying the sample comprising the target cell to the stationary phase.

9. A method of isolating a target cell from a sample that is an eluate comprising one or more unbound receptor binding reagent and a competition reagent, wherein the competition reagent disrupts binding of the receptor binding reagent to a receptor molecule, wherein the target cell has the receptor molecule on the target cell surface, the method comprising:

exposing the sample to chromatography on a stationary phase, wherein:

the sample comprises one or more target cells, a competition reagent, and an unbound receptor binding reagent, wherein the receptor binding reagent is a monovalent antibody fragment comprising (i) a monovalent binding site B capable of specifically binding to the receptor molecule and (ii) a binding partner C comprising a streptavidin binding peptide, and

the stationary phase comprises a gel filtration matrix and/or affinity chromatography matrix, wherein the gel filtration and/or affinity chromatography matrix comprises an affinity reagent comprising a streptavidin mutein, wherein the affinity reagent

comprises a plurality of binding site Z that specifically bind to the binding partner C and to the competition reagent,

incubating the sample with the stationary phase under conditions to allow binding of binding site Z with the binding partner C and with the competition reagent; and

eluting from the stationary phase at least one target cell that is free from a bound receptor binding reagent.

10. The method of claim 9, wherein the affinity chromatography matrix and/or the gel filtration matrix has the affinity reagent covalently immobilized thereon.

11. The method of any of claims 1 to 10, wherein the chromatography is column chromatography or planar chromatography.

12. A method of chromatographically isolating a target cell from a sample, wherein the target cell has a receptor molecule on the surface of the target cell, the method comprising:

providing a sample comprising one or more target cells, and a receptor binding reagent, wherein said receptor binding reagent is a monovalent antibody fragment comprising (i) a monovalent binding site B capable of specifically binding to the receptor molecule on the target cell surface with a dissociation constant ( $K_D$ ) that is of low affinity, wherein said low affinity is in the range of about  $10^{-3}$  to about  $10^{-7}$  M, or with a dissociation rate constant ( $k_{off}$ ) of about  $3 \times 10^{-5} \text{ sec}^{-1}$  or greater and (ii) a binding partner C comprising a streptavidin binding peptide;

exposing the sample to chromatography on a first stationary phase, the first stationary phase having a first affinity reagent comprising a streptavidin mutein immobilized thereon, wherein the first affinity reagent comprises a plurality of first binding site Z, and said first binding site Z is capable of forming a non-covalent reversible complex with the binding partner C and wherein the monovalent binding site B is capable of binding to the receptor molecule on the target cell surface, thereby reversibly immobilising the one or more target cell on the stationary phase,

loading a competition reagent onto the first stationary phase, the competition reagent comprising a binding site that is capable of specifically binding to the first binding site Z, wherein binding of the competition reagent to the first binding site Z allows disruption of the

non-covalent reversible complexes formed between the binding partner C of the receptor binding reagent and the first binding site Z of the affinity reagent;

recovering an elution sample from the eluate of the first stationary phase, wherein the elution sample comprises the one or more target cells, the competition reagent, and the unbound receptor binding reagent;

exposing the elution sample to chromatography on a second stationary phase, the second stationary phase being a gel filtration matrix and/or affinity chromatography matrix, wherein the gel filtration and/or affinity chromatography matrix comprises a second affinity reagent comprising a streptavidin mutein, wherein the second affinity reagent comprises a plurality of second binding sites Z that specifically bind to the binding partner C comprised in the unbound receptor binding reagent, and

passing the elution sample through the second stationary phase under conditions sufficient to allow binding of the second binding site Z with the binding partner C, thereby obtaining a sample comprising at least one target cell that is free from a bound receptor binding reagent.

13. The method of claim 12, wherein the second stationary phase is an affinity chromatography matrix or both an affinity chromatography matrix and gel filtration matrix.

14. The method of claim 12 or claim 13, wherein exposing the elution sample to chromatography on the second stationary phase comprises allowing the competition reagent to form a complex with a binding site Z of the affinity reagent, thereby immobilizing the competition reagent onto the second stationary phase.

15. The method of any of claims 12-14, wherein each of the first and the second stationary phase is comprised in a column or is a planar stationary phase.

16. The method of any of claims 9-11, wherein the dissociation constant ( $K_D$ ) for the binding between the receptor binding reagent via the monovalent binding site B to the receptor molecule is of low affinity, wherein said low affinity is in the range of about  $10^{-3}$  to about  $10^{-7}$  M, or wherein the dissociation rate constant ( $k_{off}$ ) for the binding between the receptor binding reagent via the binding site B and the receptor molecule has a value of about  $3 \times 10^{-5} \text{ sec}^{-1}$  or greater.

17. The method of any of claims 1 to 8 and 12 to 16, wherein when the  $k_{\text{off}}$  rate is of about  $3 \times 10^{-5} \text{ sec}^{-1}$  or greater, the binding between the binding site B of the receptor binding reagent and the receptor molecule has a  $k_{\text{off}}$  rate of about  $1 \times 10^{-4} \text{ sec}^{-1}$  or greater, about  $2.0 \times 10^{-4} \text{ sec}^{-1}$  or greater, about  $3 \times 10^{-4} \text{ sec}^{-1}$  or greater, about  $4 \times 10^{-4} \text{ sec}^{-1}$  or greater, about  $5 \times 10^{-4} \text{ sec}^{-1}$  or greater, about  $1 \times 10^{-3} \text{ sec}^{-1}$  or greater, about  $5 \times 10^{-3} \text{ sec}^{-1}$  or greater, about  $1 \times 10^{-2} \text{ sec}^{-1}$  or greater, about  $5 \times 10^{-2} \text{ sec}^{-1}$  or greater, about  $1 \times 10^{-1} \text{ sec}^{-1}$  or greater or about  $5 \times 10^{-1} \text{ sec}^{-1}$  or greater.

18. The method of any of claims 1 to 8 and 12 to 17, wherein when the  $k_{\text{off}}$  rate is of about  $3 \times 10^{-5} \text{ sec}^{-1}$  or greater, the binding between the binding site B of the receptor binding reagent and the receptor molecule has a  $k_{\text{off}}$  rate of about  $1 \times 10^{-2} \text{ sec}^{-1}$  or greater, about  $5 \times 10^{-2} \text{ sec}^{-1}$  or greater, about  $1 \times 10^{-1} \text{ sec}^{-1}$  or greater or about  $5 \times 10^{-1} \text{ sec}^{-1}$  or greater.

19. The method of any of claims 1-18, wherein the binding between the binding partner C of the receptor binding reagent and the binding site Z of the affinity reagent has a dissociation constant ( $K_D$ ) in the range from about  $10^{-2}$  to about  $10^{-13} \text{ M}$ .

20. The method of any of claims 12 to 19, wherein the binding between the binding partner C of the receptor binding reagent and the first binding site Z of the affinity reagent has a higher affinity than binding between the first binding site Z and the competition reagent.

21. The method of any of claims 1-20, wherein the streptavidin mutein comprises the amino acid sequence Val<sup>44</sup>-Thr<sup>45</sup>-Ala<sup>46</sup>-Arg<sup>47</sup> at sequence positions 44 to 47 of wild type streptavidin or the streptavidin mutein comprises the amino acid sequence Ile<sup>44</sup>-Gly<sup>45</sup>-Ala<sup>46</sup>-Arg<sup>47</sup> at sequence positions 44 to 47 of wild type streptavidin.

22. The method of claim 21, wherein the streptavidin mutein comprises an N-terminal amino acid residue starting at an amino acid in the region of amino acids 10 to 16 of the wildtype streptavidin amino acid sequence and an C-terminal amino acid residue ending in the region of amino acids 133 to 142 of the wildtype streptavidin amino acid sequence.

23. The method of any of claims 1-22, wherein the streptavidin binding peptide comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO:3).

24. The method of any of claims 1-23, wherein the streptavidin binding peptide comprises two or more individual binding modules comprising the sequence set forth by SEQ ID NO:3.

25. The method of any of claims 1-24, wherein the streptavidin-binding peptide comprises the sequence SAWSHPQFEK(GGGS)<sub>2</sub>GGSAWSHPQFEK (SEQ ID NO:13).

26. The method of any of claims 4-25, wherein the competition reagent is biotin or a biotin analogue.

27. The method of any of claims 1 to 26, wherein the stationary phase is a non-magnetic material or non-magnetisable material.

28. The method of claim 27, wherein the stationary phase comprises or consists of one of a cellulose membrane, a plastic membrane, a polysaccharide gel, a polyacrylamide gel, an agarose gel, polysaccharide grafted silica, polyvinylpyrrolidone grafted silica, polyethylene oxide grafted silica, poly(2-hydroxyethylaspartamide) silica, poly(N- isopropylacrylamide) grafted silica, a styrene-divinylbenzene gel, a copolymer of an acrylate or an acrylamide and a diol, a co-polymer of a polysaccharide and N,N'- methylenebisacrylamide and a combination of any two or more thereof.

29. The method of any of claims 9 to 28, wherein the affinity chromatography matrix and/or the gel filtration matrix comprises or consists of a monolithic matrix, a particulate matrix, or a planar matrix.

30. The method of claim 29, wherein the particulate matrix has a mean particle size of about 5 µm to about 200 µm or of about 5 µm to 600 µm, or of about 5µm to 1500µm.

31. The method of any of claims 9-30, wherein the affinity chromatography matrix and/or the gel filtration matrix has a mean pore size of 0 to about 500 nm.

32. The method of any of claims 1 to 31, wherein the receptor binding reagent is selected from the group consisting of a Fab fragment, a Fv fragment or a single chain Fv fragment.

33. The method of any of claims 1 to 32, wherein the sample is or comprises a fluid, optionally wherein the fluid is a body fluid, optionally is blood or a blood component.

34. The method of any of claims 1 to 33, wherein the target cell is a mammalian cell.

35. The method of any of claims 1 to 34, wherein the target cell is a leukocyte or a stem cell, optionally wherein the leukocyte is a lymphocyte.

36. A kit of parts for isolating a target cell, wherein the target cell has a receptor molecule on the target cell surface, the kit comprising:

(a) a receptor binding reagent that is a monovalent antibody fragment comprising a monovalent binding site B and a binding partner C, wherein the monovalent binding site B is capable of specifically binding to the receptor molecule on the target cell surface with a dissociation constant ( $K_D$ ) that is of low affinity, wherein said low affinity is in the range of about  $10^{-3}$  to about  $10^{-7}$  M, or with a dissociation rate constant ( $k_{off}$ ) of about  $3 \times 10^{-5} \text{ sec}^{-1}$  or greater, and wherein the binding partner C comprises a streptavidin binding peptide capable of reversibly binding to a binding site Z of an affinity reagent; and

(b) a stationary phase suitable for cell separation, the stationary phase having the affinity reagent comprising a streptavidin mutein immobilized thereon, wherein the affinity reagent comprises the binding site Z.

37. The kit of parts of claim 36, wherein the receptor binding reagent is immobilized on the stationary phase by a non-covalent reversible complex formed by binding to the binding site Z of the affinity reagent.

38. The kit of parts of claim 36 or claim 37, wherein the stationary phase is comprised in a chromatography column or is a planar stationary phase.

39. The kit of parts of any of claims 36 to 38, comprising a second stationary phase suitable for cell separation from other components in a sample, the second stationary phase having an affinity reagent comprising a streptavidin mutein immobilized thereon, wherein the affinity reagent comprises a binding site Z capable of specifically binding to the binding partner C comprised in the receptor binding reagent.

40. The kit of parts of claim 39, wherein the second stationary phase is comprised in a chromatography column or is a planar stationary phase.

41. The kit of parts of any of claims 36-40, wherein when the  $k_{off}$  rate is of about  $3 \times 10^{-5} \text{ sec}^{-1}$  or greater, the binding between the binding site B of the receptor binding reagent and the receptor molecule has a  $k_{off}$  rate of about  $1 \times 10^{-4} \text{ sec}^{-1}$  or greater, about  $2.0 \times 10^{-4} \text{ sec}^{-1}$  or greater, about  $3 \times 10^{-4} \text{ sec}^{-1}$  or greater, about  $4 \times 10^{-4} \text{ sec}^{-1}$  or greater, about  $5 \times 10^{-4} \text{ sec}^{-1}$  or greater, about  $1 \times 10^{-3} \text{ sec}^{-1}$  or greater, about  $5 \times 10^{-3} \text{ sec}^{-1}$  or greater, about  $1 \times 10^{-2} \text{ sec}^{-1}$  or

greater, about  $5 \times 10^{-2} \text{ sec}^{-1}$  or greater, about  $1 \times 10^{-1} \text{ sec}^{-1}$  or greater or about  $5 \times 10^{-1} \text{ sec}^{-1}$  or greater.

42. The kit of parts of any of claims 36-41, wherein when the  $k_{\text{off}}$  rate is of about  $3 \times 10^{-5} \text{ sec}^{-1}$  or greater, the binding between the binding site B of the receptor binding reagent and the receptor molecule has a  $k_{\text{off}}$  rate of about  $1 \times 10^{-2} \text{ sec}^{-1}$  or greater, about  $5 \times 10^{-2} \text{ sec}^{-1}$  or greater, about  $1 \times 10^{-1} \text{ sec}^{-1}$  or greater or about  $5 \times 10^{-1} \text{ sec}^{-1}$  or greater.

43. The kit of parts of any of claims 36-42, wherein the receptor binding reagent is selected from the group consisting of a Fab fragment, a Fv fragment or a single chain Fv fragment.

44. The kit of parts of any of claims 36-43, wherein the streptavidin mutein comprises the amino acid sequence Val<sup>44</sup>-Thr<sup>45</sup>-Ala<sup>46</sup>-Arg<sup>47</sup> at sequence positions 44 to 47 of wild type streptavidin or the streptavidin mutein comprises the amino acid sequence Ile<sup>44</sup>-Gly<sup>45</sup>-Ala<sup>46</sup>-Arg<sup>47</sup> at sequence positions 44 to 47 of wild type streptavidin.

45. The kit of parts of claim 44, wherein the streptavidin mutein comprises an N-terminal amino acid residue starting at an amino acid in the region of amino acids 10 to 16 of the wildtype streptavidin amino acid sequence and an C-terminal amino acid residue ending in the region of amino acids 133 to 142 of the wildtype streptavidin amino acid sequence.

46. The kit of parts of any of claims 36-45, wherein the streptavidin binding peptide comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO:3).

47. The kit of parts of any of claims 36-46, wherein the streptavidin binding peptide comprises two or more individual binding modules comprising the sequence set forth by SEQ ID NO:3.

48. The kit of parts of any of claims 36-47, wherein the streptavidin-binding peptide comprises the sequence SAWSHPQFEK(GGGS)<sub>2</sub>GGSAWSHPQFEK (SEQ ID NO:13).

49. An apparatus for purification of target cells, the apparatus comprising:

at least one of a first and a second stationary phase for chromatography wherein:

the first stationary phase is suitable for cell separation, the first stationary phase comprising an affinity chromatography matrix having a first affinity reagent comprising a

streptavidin mutein covalently immobilized thereon, wherein the first affinity reagent has a plurality of binding site Z capable of reversibly binding to a binding partner C comprised in a first receptor binding reagent, wherein the receptor binding reagent is a monovalent antibody fragment further comprising a monovalent binding site B capable of specifically binding to the receptor molecule on the target cell surface with a dissociation constant ( $K_D$ ) that is of low affinity, wherein said low affinity is in the range of about  $10^{-3}$  to about  $10^{-7}$  M, or with a dissociation rate constant ( $k_{off}$ ) of about  $3 \times 10^{-5} \text{ sec}^{-1}$  or greater, and wherein the binding partner C comprises a streptavidin binding peptide; and

the second stationary phase is suitable for cell separation, the second stationary phase is a gel filtration matrix and/or affinity chromatography matrix having a second affinity reagent comprising a streptavidin mutein immobilized thereon, wherein the second affinity reagent has a plurality of binding site Z capable of specifically binding to said binding partner C.

50. The apparatus of claim 49, wherein each the first and the second stationary phase is either comprised in a chromatography column or is a planar stationary phase.

51. The apparatus of claim 49 or 50, further comprising a plurality of arrangements of first and second stationary phases being fluidly connected in series.

52. The apparatus of claim 51, comprising a sample inlet being fluidly connected to the first stationary phase of the first arrangement of a first and second stationary phases for chromatography.

53. The apparatus of claim 52, comprising a sample outlet for purified target cells, the sample outlet being fluidly connected to the second stationary phase of the last of the at least one arrangement of a first and second stationary phases for chromatography.

54. The apparatus of any of claims 49-53, comprising a competition reagent container that is fluidly connected to at least one of the first stationary phases of the arrangements of a first and second stationary phases for chromatography.

55. The apparatus of claim 54, wherein the competition reagent container comprises a competition reagent that is biotin or a biotin analogue.

56. The apparatus of any of claims 49-55, wherein the streptavidin mutein comprises the amino acid sequence Val<sup>44</sup>-Thr<sup>45</sup>-Ala<sup>46</sup>-Arg<sup>47</sup> at sequence positions 44 to 47 of

wild type streptavidin or the streptavidin mutein comprises the amino acid sequence Ile<sup>44</sup>-Gly<sup>45</sup>-Ala<sup>46</sup>-Arg<sup>47</sup> at sequence positions 44 to 47 of wild type streptavidin.

57. The apparatus of claim 56, wherein the streptavidin mutein comprises an N-terminal amino acid residue starting at an amino acid in the region of amino acids 10 to 16 of the wildtype streptavidin amino acid sequence and an C-terminal amino acid residue ending in the region of amino acids 133 to 142 of the wildtype streptavidin amino acid sequence.

58. The apparatus of any of claims 49-57, wherein the streptavidin binding peptide comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO:3).

59. The apparatus of any of claims 49-58, wherein the streptavidin binding peptide comprises two or more individual binding modules comprising the sequence set forth by SEQ ID NO:3.

60. The apparatus of any of claims 49-59, wherein the streptavidin-binding peptide comprises the sequence SAWSHQPFEK(GGGS)<sub>2</sub>GGSAWSHQPFEK (SEQ ID NO:13).