Claims

1. A method for separating viable target cells from a sample comprising the steps of:
   a. contacting a sample comprising a suspension of viable target cells that display a molecule on the cell surface with non-porous microparticles that have a density of about 1.45 g/cm^3 or greater, a diameter of about 10µm to 200µm, and a capture ligand covalently immobilized to the microparticle surface that is capable of specifically binding to said molecule;
   b. incubating said sample without substantial agitation to form a target cell/microparticle complex;
   c. separating non-bound substances in said sample from said target cell/microparticle complex by washing said non-bound substances through a filter while retaining said target cell/microparticle complex;
   d. mechanically dissociating said target cell/microparticle complex and eluting said viable target cells through said filter while retaining said microparticles with said capture ligand covalently immobilized to the microparticle surface.

2. The method according to claim 1, wherein the target cells are suitable for cell therapy.

3. The method according to claim 1 or claim 2, wherein the capture ligand specifically binds to said molecule at the surface of said target cell with a dissociation constant between 10^{-5} and 10^{-12}, more preferably between 10^{-9} and 10^{-11} or more preferably between 10^{-7} and 10^{-10} and more preferably is in the order of about 10^{-9}.

4. The method according to any one of claims 1 to 3, wherein the capture ligand is selected from the group of an antibody, a $FabFc_2$, a Fab, a Fv, a Fd, a $F(ab')_2$, an Fv fragment containing only the light and heavy chain variable regions, a Fab or $F(ab')_2$ fragment containing the variable regions and parts of the constant regions, a single-chain antibody, a scFv, a CDR-grafted antibody, a dAb and or a nanobody.

5. The method according to any one of claims 1 to 4, wherein said cell surface molecule is selected from the group of human CD2, CD3, CD4, CD8, CD11a, CD11b, CD14, CD15, CD16, CD19, CD20, CD22, CD24, CD25, CD27, CD30, CD31, CD34, CD38, CD43, CD45, CD48, CD56, CD61, CD73, CD90, CD91, CD105, CD114, CD117, CD140b, CD150, CD182, CD184, CD271, CDCP1, GD2, GPR4, Sca-1, and STRO-1.

6. The method according to any one of claims 1 to 5, wherein the method further comprises obtaining a medicament comprising a viable target cell suspension of the eluted viable target cells, wherein the eluted viable target cell suspension is essentially free from capture ligand.

7. The method according to any one of claims 1 to 6, wherein the target cell is selected from the group of human granulocytes, T lymphocytes, monocytes, T regulatory cells, T helper cells, cytotoxic T cells, B lymphocytes, tumour infiltrating lymphocytes, thrombocytes,

natural killer cells, hematopoietic stem and progenitor cells, mesenchymal/stromal stem cells, hair follicle stem cells, cardiac stem cells, multipotent muscle cells, neural stem cells, hepatic stem cells, dental pulp cells, periodontal ligament cells, retinal pigment epithelial cells, adipose-derived stem and progenitor cells, pluripotent stem cells including embryonic stem cells and induced pluripotent stem cells, Car-T cells, Microvascular endothelial cells (MVEC), primary epithelial cells such as keratinocytes, cervical epithelial cells, bronchial epithelial cells, tracheal epithelial cells, kidney epithelial cells and retinal epithelial cells, fibroblast cells from any tissue or organ including heart, liver, kidney, colon, intestine, esophagus, stomach, neural tissue derived from the brain and spinal cord, lung, vascular tissue derived from artery, vein and capillary, lymphoid tissue derived from lymph gland, adenoid, tonsil, bone marrow, and blood, spleen, muscle cells, pancreatic cells, cardiac cells and cells established from such primary cells.

8. The method according to any one of claims 1 to 7, wherein the target cell is selected from the group of monocytes, T lymphocytes, B lymphocytes, CAR-T cells and stem cells.

9. The method according to any one of claims 1 to 8, wherein said sample is selected from the group of human whole blood, apheresis, bone marrow aspirate, biopsy, liquified tissue such as liposuction that have been enzymatically degraded, cell culture, bioreactor culture, tumour cells, single cell suspension.

10. A closed housing cartridge configured to be used in the separation of viable target cells from a sample according to the method according to any one of claims 1 to 9.

11. The closed housing cartridge according to claim 10, comprising:
    -a filter enclosed within a unit with an inlet port and an outlet port,
    -a fluid conduit that is connected to the filter inlet and that has one or more valves that are capable of regulating the flow of a source of a sample comprising target cells, a source of a suspension of the non-porous microparticles, a source of wash solution, and a source of elution solution, and
    -a fluid conduit connected to the filter outlet that comprises one or more valves wherein one valve is capable of diverting a solution of target cells via a fluid conduit to a container for collecting target cells or to a different container for wash solutions and non-bound substances.

12. A kit-of-parts comprising the closed housing cartridge according to claim 10 or 11 and a container comprising a suspension of non-porous microparticles that have a density of about 1.45 g/cm^3 or greater, a diameter of about 10µm to 200µm, and a capture ligand covalently immobilized to the microparticle surface that is capable of specifically binding to a molecule on the surface of a cell.

13. The closed housing cartridge according to claim 10 or 11 or the kit-of-parts according to claim 12, wherein the closed housing cartridge or the components of the kit-of-parts are contained within a sealed sterile package.

14. The method according to any one of claims 1 to 9 or the kit-of-parts according to claim 12 or 13, wherein said non-porous microparticles have a density of about 1.95 g/cm^3 or more.

15. The method according to any one of claims 1 to 9 and 14 or the kit-of-parts according to any one of claims 12 to 14, wherein said non-porous microparticles have a diameter of about 35µm to 50µm.

16. The method according to any one of claims 1 to 9, 14 and 15 or the kit-of-parts according to any one of claims 12 to 15, wherein said non-porous microparticles are made of silicon dioxide (SiO_2).

17. The method according to any one of claims 1 to 9, wherein the eluted viable target cell suspension is essentially free from capture ligand antibody that specifically binds to a molecule that is displayed on the target cell surface.

18. An apparatus configured to separate viable target cells from a sample according to the method of any one of claims 1 to 9 comprising:
    -a means for securely mounting the closed housing cartridge according to claim 10 or 11 such that the valves of the cartridge can be mechanically operated by an electronic control system,
    -a means for connecting the cartridge to one or more containers with one or more solutions and/or suspensions,
    -a means for moving solutions and/or suspensions, and
    -a control system configured to set, measure, and/or adjust the flow of solutions and/or suspensions via the valves of the cartridge while the method according to any one of claims 1 to 9 is performed.

19. The apparatus according to claim 18, wherein said apparatus comprises one or more pre-treatment filters, keyboards, touch screens, bar code reading devices, communication elements for WIFI, near field communication (NFC) or Bluetooth, motors, vibration isolation units for any or all pumps or motors, sensors or electrodes that measure pressure, flow, impedance, conductivity, resistivity, electric current, pH, temperature, humidity, gas percentages, radiation, motion, volume, weight, magnetic flux, time, turbidity, optical density, alarms, cell counting devices, heating units, cooling units, graphical display units, ports for data storage devices such as CDs, USB flash drive sticks or NFC.

20. A medicament comprising a viable target cell suspension obtainable according to the method of any one of claims 6 to 9, wherein the eluted viable target cell suspension is essentially free from capture ligand.

21. A viable target cell suspension obtainable according to the method of any one of claims 6 to 9 for use as a medicament, wherein the eluted viable target cell suspension is essentially free from capture ligand.

22. Use of a viable target cell suspension obtainable according to the method of any one of claims 6 to 9 in the manufacture of a medicament for prevention and/or treatment of disease in a subject, wherein said viable target cell suspension that is essentially free from capture ligand is to be administered to said subject.