

**Claims:**

Claim 1. A method for evaluating the expression of one or more molecules of interest by cell types of interest within a heterogeneous population of cells, comprising:

- 5 (i) sorting cells of the heterogeneous population of cells into two or more cell types based on expression of one or more cell type markers by the cells, wherein the heterogeneous population of cells is sorted into the two or more cell types by fluorescence activated cell sorting (FACS);
- (ii) labelling the cells with target-binding molecules capable of binding to one or more molecules of interest, comprising labelling the different sorted cell types with target-binding molecules capable of
- 10 binding to non-identical molecules of interest; and
- (iii) analysing the cells in order to determine expression of the one or more molecules of interest by the one or more cell types of interest.

Claim 2. The method according to claim 1, wherein the method further comprises labelling the sorted cell

15 types with one or more cell type-specific identifiers prior to analysis at step (iii).

Claim 3. The method according to claim 1 or claim 2, wherein the method comprises mixing together the sorted, labelled cells of different cell types prior to analysis at step (iii).

20 Claim 4. The method according to any one of claims 1 to 3, wherein labelling the cells with target-binding molecules capable of binding to one or more molecules of interest comprises: labelling the cells with target-binding molecules capable of binding to one or more molecules of interest using target-binding molecules conjugated to non-identical elemental isotopes, or labelling the cells with target-binding molecules capable of binding to one or more molecules of interest using target-binding molecules

25 conjugated to non-identical fluorophores.

Claim 5. The method according to any one of claims 2 to 4, wherein labelling the sorted cell types with one or more cell type-specific identifiers comprises: labelling the different sorted cell types using target-binding molecules conjugated to non-identical elemental isotopes, or labelling the different sorted cell

30 types using target-binding molecules conjugated to non-identical fluorophores.

Claim 6. The method according to any one of claims 1 to 5, wherein analysing the cells in order to determine expression of the one or more molecules of interest by the cell types comprises analysis by mass cytometry or analysis by flow cytometry.

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Claim 7. A method for evaluating the expression of one or more molecules of interest by one or more cell types of interest within a heterogeneous population of cells, comprising:

- (i) sorting cells of the heterogeneous population of cells into two or more cell types based on expression of one or more cell type markers by the cells as determined by flow cytometry, wherein the

heterogeneous population of cells is sorted into the two or more cell types by fluorescence activated cell sorting (FACS);

(ii) labelling the cells with target-binding molecules capable of binding to one or more molecules of interest, comprising labelling the different sorted cell types with target-binding molecules capable of binding to non-identical molecules of interest using target-binding molecules conjugated to non-identical elemental isotopes; and

(iii) analysing the cells by mass cytometry in order to determine expression of the one or more molecules of interest by the one or more cell types of interest.

Claim 8. The method according to claim 7, wherein the method further comprises labelling the sorted cell types with target-binding molecules conjugated to an elemental isotope, wherein the different sorted cell types are labelled with target-binding molecules conjugated to non-identical elemental isotopes prior to analysis at step (iii).

Claim 9. A method for evaluating the expression of one or more molecules of interest by one or more cell types of interest within a heterogeneous population of cells, comprising:

(i) sorting cells of the heterogeneous population of cells into two or more cell types based on expression of one or more cell type markers by the cells as determined by flow cytometry, wherein the heterogeneous population of cells is sorted into the two or more cell types by fluorescence activated cell sorting (FACS);

(ii) labelling the cells with target-binding molecules capable of binding to one or more molecules of interest, comprising labelling the different sorted cell types with target-binding molecules capable of binding to non-identical molecules of interest using target-binding molecules conjugated to non-identical fluorophores; and

(iii) analysing the cells by flow cytometry in order to determine expression of the one or more molecules of interest by the one or more cell types of interest.

Claim 10. The method according to claim 9, wherein the method further comprises labelling the sorted cell types with target-binding molecules conjugated to a fluorophores, wherein the different

sorted cell types are labelled with target-binding molecules conjugated to non-identical fluorophores prior to analysis at step (iii).

Claim 11. The method according to any one of claims 7 to 10, wherein the method comprises mixing together the sorted, labelled cells of different cell types prior to analysis at step (iii).

Claim 12. The method according to any one of claims 1 to 11, wherein the method comprises analysing two or more heterogeneous populations of cells, and additionally comprises labelling one or more of the heterogeneous populations of cells with a population-specific identifier.

Claim 13. A method for evaluating the expression of one or more molecules of interest by one or more cell types of interest within two or more heterogeneous populations of cells, comprising:

(i) labelling the cells of one or more of the heterogeneous populations of cells with a population-specific identifier;

(ii) sorting cells of the heterogeneous populations of cells into two or more cell types based on expression of one or more cell type markers by the cells, wherein the heterogeneous population of cells is sorted into the two or more cell types by fluorescence activated cell sorting (FACS);

(iii) labelling the cells with target-binding molecules capable of binding to one or more molecules of interest, comprising labelling the different sorted cell types with target-binding molecules capable of binding to non-identical molecules of interest; and

(iv) analysing the cells in order to determine expression of the one or more molecules of interest by the one or more cell types of interest.

Claim 14. The method according to claim 13, wherein the method further comprises labelling the sorted cell types with one or more cell type-specific identifiers prior to analysis at step (iv).

Claim 15. The method according to any one of claims 13 or claim 14, wherein the method comprises mixing together the sorted, labelled cells of different cell types prior to analysis at step (iv).

Claim 16. The method according to any one of claims 13 or claim 14, wherein the method comprises mixing together sorted, labelled cells of the same type from the two or more heterogeneous populations of cells prior to analysis at step (iv).

Claim 17. The method according to any one of claims 1 to 16, wherein the method additionally comprises mixing the labelled cell types with buffer cells.

Claim 18. The method according to claim 17, wherein the buffer cells are labelled with target-binding molecules conjugated to magnetic beads.

Claim 19. The method according to claim 17 or claim 18, wherein the method additionally comprises separating the buffer cells from cells of the heterogeneous population of cells prior to analysing the cells in order to determine expression of the one or more molecules of interest.

Claim 20. The method according to any one of claims 1 to 19, wherein the heterogeneous population of cells is a population of immune cells.

Claim 21. The method according to any one of claim 1 to 20, wherein the heterogeneous population of cells is a population of peripheral blood mononuclear cells (PBMCs).