

CLAIMS

1. A method for the isolation of lymphocytes from a non-haematopoietic tissue sample comprising the steps of:
 - (i) culturing the non-haematopoietic tissue sample in the presence of Interleukin-1 β (IL-1 β); and
 - (ii) collecting a population of lymphocytes cultured from the non-haematopoietic tissue sample.
2. A method for the isolation of $\gamma\delta$ T cells from a non-haematopoietic tissue sample comprising the steps of:
 - (i) culturing the non-haematopoietic tissue sample in the presence of IL-1 β ; and
 - (ii) collecting a population of $\gamma\delta$ T cells cultured from the non-haematopoietic tissue sample.
3. The method according to any preceding claim, wherein step (i) further comprises culturing the non-haematopoietic tissue sample in the presence of Interleukin-2 (IL-2) and Interleukin-15 (IL-15).
4. The method according to any preceding claim, wherein step (i) further comprises culturing the non-haematopoietic tissue sample in the presence of Interleukin-4 (IL-4) and/or Interferon- γ (IFN- γ).
5. The method according to any preceding claim, wherein the culturing is performed in the absence of Interleukin-21 (IL-21).
6. The method according to any one of claims 1 to 4, wherein the culturing is performed in the presence Interleukin-21 (IL-21) at a concentration between 15 ng/mL and 25 ng/mL, such as 18 to 20 ng/ml.
7. The method according to any preceding claim, wherein the non-haematopoietic tissue sample is cultured in media containing serum or plasma.
8. The method according to claim 7, wherein the non-haematopoietic tissue is cultured in media containing 2.5% human plasma.
9. The method according to claim 7, wherein the non-haematopoietic tissue is cultured in media containing 10% human AB serum.

10. The method according to any preceding claim, wherein the lymphocytes or $\gamma\delta$ T cells are collected after at least 7 days of culturing.
11. The method according to any preceding claim, wherein the lymphocytes or $\gamma\delta$ T cells are collected after at least 14 days of culturing.
12. The method according to any preceding claim, wherein the lymphocytes or $\gamma\delta$ T cells are collected prior to 35 days of culturing.
13. The method according to any preceding claim, wherein the lymphocytes or $\gamma\delta$ T cells are collected after 19 days of culturing.
14. The method according to any preceding claim, wherein the lymphocytes or $\gamma\delta$ T cells are collected after 21 days of culturing.
15. The method according to any preceding claim, wherein the non-haematopoietic tissue sample is an intact biopsy.
16. The method according to any preceding claim, wherein the non-haematopoietic tissue sample is skin.
17. The method according to claim 16, wherein the skin sample comprises the epidermal and dermal layer.
18. The method according to any preceding claim, wherein the non-haematopoietic tissue sample is gut or gastrointestinal tract.
19. The method according to any preceding claim, wherein the non-haematopoietic tissue sample has been obtained from a human.
20. The method according to any preceding claim, wherein the method is performed in a vessel comprising a gas permeable material.
21. The method according to any preceding claim, wherein the isolated population of lymphocytes or $\gamma\delta$ T cells comprise a population of V δ 1 T cells.

22. The method according to claim 21, wherein less than 80% of the isolated population of V δ 1 T cells expresses CD45RA, such as less than 30% of the isolated population of V δ 1 T cells expresses CD45RA.
23. The method according to claim 21 or claim 22, wherein less than 10% of the isolated population of V δ 1 T cells expresses NKG2A.
24. The method according to any preceding claim, further comprising expanding the isolated population of lymphocytes or $\gamma\delta$ T cells.
25. A method for the isolation of lymphocytes from a non-haematopoietic tissue sample comprising the steps of:
- (i) culturing the non-haematopoietic tissue sample in media comprising serum replacement, human plasma and/or human AB serum; and
 - (ii) collecting a population of lymphocytes cultured from the non-haematopoietic tissue sample.
26. The method according to claim 25, comprising culturing the non-haematopoietic tissue sample in media comprising serum replacement.
27. The method according to either claim 25 or 26, wherein the isolated population of lymphocytes comprise a population of TCR-negative cells.
28. The method according to any one of claims 25 to 27, wherein step (i) further comprises culturing the non-haematopoietic tissue sample in the presence of IL-2 and IL-15.
29. The method according to any one of claims 25 to 28, wherein step (i) further comprises culturing the non-haematopoietic tissue sample in the presence of IL-4 and/or IL-1 β .
30. The method according to any one of claims 25 to 29, wherein the culturing is performed in the absence of IFN- γ .
31. The method according to any one of claims 25 to 30, wherein step (i) further comprises culturing the non-haematopoietic tissue sample in the presence of IL-21 at a concentration between 15 ng/mL and 25 ng/mL.
32. The method according to any one of claims 25 to 31, wherein the lymphocytes are collected after 19 days of culturing.

33. The method according to any one of claims 25 to 31, wherein the lymphocytes are collected after 21 days of culturing.

34. A method for the isolation and expansion of lymphocytes from a non-haematopoietic tissue sample comprising the steps of:

(i) isolating a population of lymphocytes from the non-haematopoietic tissue sample according to the method of any preceding claim; and

(ii) further culturing said population of lymphocytes for at least 5 days to produce an expanded population of lymphocytes.

35. A method for the isolation and expansion of $\gamma\delta$ T cells from a non-haematopoietic tissue sample comprising the steps of:

(i) isolating a population of $\gamma\delta$ T cells from the non-haematopoietic tissue sample according to the method of any preceding claim; and

(ii) further culturing said population of $\gamma\delta$ T cells for at least 5 days to produce an expanded population of $\gamma\delta$ T cells.

36. The method according to claim 34 or claim 35, wherein the isolated lymphocyte or $\gamma\delta$ T cell population is frozen and then thawed prior to step (ii).

37. The method according to claim 35 or claim 36, wherein the expansion step comprises culturing the $\gamma\delta$ T cells in the presence of:

(a) IL-2 or IL-9;

(b) IL-15; and optionally

(c) IL-21

for at least 5 days in amounts effective to produce an expanded population of $\gamma\delta$ T cells.

38. The method according to claim 37, which further comprises culturing the $\gamma\delta$ T cells in the presence of IL-4.

39. The method according to any one of claims 35 to 38, wherein the expansion step comprises culturing the $\gamma\delta$ T cells in the absence of substantial stromal cell contact.

40. The method according to any one of claims 35 to 39, wherein the expansion step comprises the absence of exogenous TCR pathway agonists.

41. A method of isolation for $\gamma\delta$ T cells from a non-haematopoietic tissue sample comprising the steps of:
- (i) isolating a population of $\gamma\delta$ T cells from the non-haematopoietic tissue sample; and
 - (ii) freezing the isolated population of $\gamma\delta$ T cells.
42. The method according to claim 41, wherein $\gamma\delta$ T cells are isolated by culturing the non-haematopoietic tissue sample in the presence of IL-2 and IL-15, optionally in combination with IL-1 β , IL-4 and/or IL-21.
43. The method according to claim 42, wherein $\gamma\delta$ T cells are isolated by culturing the non-haematopoietic tissue sample in the presence of IL-21 at a concentration between 15 ng/mL and 25 ng/mL, such as 18 to 20 ng/mL.
44. The method according to claim 41 to 43, wherein the $\gamma\delta$ T cells are frozen after 19 days of isolation.
45. The method according to any one of claims 41 to 43, wherein the $\gamma\delta$ T cells are frozen after 21 days of isolation.
46. The method according to any one of claims 41 to 44, wherein the isolated population of $\gamma\delta$ T cells are frozen in a freezing solution and stored in liquid nitrogen (vapour phase).
47. The method according to any one of claims 41 to 45, which additionally comprises: (iii) thawing the frozen population of $\gamma\delta$ T cells; and (iv) culturing the thawed population of $\gamma\delta$ T cells for at least 5 days to produce an expanded population of $\gamma\delta$ T cells.
48. An isolated $\gamma\delta$ T cell population obtainable by the method of any one of claims 1 to 24.
49. An isolated $\gamma\delta$ T cell population obtained by the method of any one of claims 1 to 24.
50. A frozen isolated $\gamma\delta$ T cell population obtainable by the method of any one of claims 41 to 47.
51. A frozen isolated $\gamma\delta$ T cell population obtained by the method of any one of claims 41 to 47.
52. An isolated and expanded $\gamma\delta$ T cell population obtainable by the method of any one of claims 35 to 40.

52. An isolated and expanded $\gamma\delta$ T cell population obtained by the method of any one of claims 35 to 40.