CLAIMS

1. A method for producing an expanded population of activated human T regulatory (Treg) cells that are at least about 60% $CD4^+CD25^+CD45RA^+CD45RO^+$ and less than or equal to about 10% $CD4^-CD8^+$ from at least one cryopreserved human umbilical cord blood unit, the method comprising:

a) thawing the at least one cryopreserved human umbilical cord blood unit in a single step in a water bath;

b) diluting and washing the thawed umbilical cord blood unit in a solution comprising PBS, EDTA, magnesium chloride, and 0.5% human serum albumin in a functionally closed system without manual washing, thereby producing washed cord blood cells;

c) isolating naturally occurring Treg cells using a double selection method based on $CD25^+$ cell surface expression using a double ferromagnetic column method, wherein the washed cord blood cells are contacted by a reagent that specifically binds to CD25 and passed over a ferromagnetic column to produce a first $CD25^+$ fraction, and the first $CD25^+$ fraction is passed over the ferromagnetic column to produce isolated $CD25^+$ Treg cells;

d) ex-vivo expanding the isolated $CD25^+$ Treg cells in a culture medium(s), in a gas permeable cultureware, in the presence of about 1000 IU/ml of interleukin-2 (IL-2) and in the presence of either (i) beads coated with an anti-CD3 antibody or an antigen-binding fragment thereof and with an anti-CD28 antibody or an antigen-binding fragment thereof, or (ii) beads coated with an anti-CD3 antibody or an antigen binding fragment thereof and beads coated with an anti-CD28 antibody or antigen binding-fragment thereof, for up to 10 days, up to 12 days or up to 14 days, wherein the culture medium is replaced about every 48 hours, to produce a population of activated $CD25^+$ Treg cells and the beads are at a 1:1 ratio and the culture medium is replaced about every 48 hours, to produce a population of activated $CD25^+$ Treg cells, wherein the entire expanding step is performed in a gas permeable cultureware;

wherein the culture is not rocked or agitated; and

e) harvesting the activated $CD25^+$ cells from the culture medium to produce an expanded population of activated human Treg cells that are at least about 60% $CD4^+CD25^+CD45RA^+CD45RO^+$ and less than or equal to about 10% $CD4^-CD8^+$.

2. The method of claim 1, wherein a single umbilical cord blood unit is used.

3. The method of claim 1 or 2, wherein between two and four pooled umbilical cord

blood units are used.

4. The method of any one of claims 1-3, wherein the reagent that specifically binds to CD25 is an anti-CD25 antibody or an antigen-binding fragment thereof.

5. The method of any one of claims 1-4, wherein the reagent that specifically binds to CD25 is conjugated to a solid support.

6. The method of claim 5, wherein the solid support is a magnetic microbead.

7. The method of any one of claims 1-6, wherein the beads coated with an anti-CD3 antibody or antigen-binding fragment thereof and the beads coated with an anti-CD28 antibody or antigen-binding fragment thereof are at a 1:1 ratio.

8. The method of any one of claims 1-7, wherein the $CD25^+$ Treg cells isolated in step c) are suspended in a culture medium comprising IL-2 at the immediate beginning of step d).

9. The method of any one of claims 1-8, wherein in step d), about 1×10^6 $CD25^+$ cells/ml are cultured.

10. The method of any one of claims 1-9, wherein in step d), the cells are initially cultured in gas-permeable cultureware that has a membrane surface area of 10 cm^2.

11. The method of claim 10, wherein the culture is subsequently transferred to gas-permeable cultureware that has a membrane surface area of 100 cm^2.

12. The method of any one of claims 1-11, wherein from 1×10^9 to 2×10^9 activated $CD25^+$ cells are harvested following 14 days of culture.

13. The method of any one of claims 1-12, wherein the expanded population of activated human Treg cells exhibit high expression of FOXP3 and low expression of RORγt.

14. The method of any one of claims 1-13, wherein the expanded population of activated

human Treg cells is at least about 95% $CD45RA^+$ and at least about 80% CD45RO.