

having a weight average molecular weight (M_w) of between 300 and 1,650 Da based on the total weight of dextrin, dextran, isomaltooligosaccharide, and derivatives thereof in said agent, or b) wherein said cryoprotectant has a weight average molecular weight (M_w) of between 300 and 9,500 Da such as between 300 and 7,500 Da, or c) wherein said cryoprotecting agent comprises at least 1% w/w of one or more of isomaltooligosaccharide and derivatives thereof having a weight average molecular weight (M_w) of between 300 and 1,650 Da based on the total weight of dextrin, dextran, isomaltooligosaccharide and derivatives thereof in said agent, and said cryoprotectant has a weight average molecular weight (M_w) of between 300 and 9,500 Da, is very useful as a cryoprotectant. Compared to the previously used dextran materials of higher molecular weight such as Dextran 40 (40,000 Da) and Dextran 70 (70,000 Da), the cryoprotecting agent comprising above described cryoprotectant has a lower viscosity due to the lower molecular weight and may thus be pre-prepared in a high concentration making it possible to add a sample already in a solution and still obtain a composition comprising both cryoprotectant and sample in a concentration suitable for cryopreservation. Furthermore, molecules with lower molecular weight are generally less immunogenic than molecules with high molecular weight. Dextran 1 is known as a hapten inhibitor that reduces the risk for anaphylactic reactions when administering dextran and is thus used as pre-injection before injection of dextrans with higher molecular weight such as Dextran 40 (40,000 Da) and Dextran 70 (70,000 Da). Dextran 1 has also been documented in studies by Richter et al (Int. Arch. Allergy 43:252-268 (1972) and Int. Arch. Allergy 41:826-844 (1971)) to have a very low immunological potential in humans. Furthermore, it has been shown in the examples that cryopreservation with the cryoprotectant as described herein provides a better protection of functionality after cryopreservation measured as viability of the tested cells than with dextrans having a higher molecular weight.

In one aspect, there is provided the use of a cryoprotecting agent comprising one or more cryoprotectants selected from the group consisting of dextrin, dextran, isomaltooligosaccharide, and derivatives thereof which derivative is selected from the group consisting of hydrogenated isomaltooligosaccharide, hydrogenated dextran, hydrogenated dextrin, oxidised isomaltooligosaccharide, oxidised dextran, oxidised dextrin, DEAE-dextrin, DEAE-dextran, DEAE-isomaltooligosaccharides, carboxyC₁₋₁₀alkyl-dextrin, carboxyC₁₋₁₀alkyl-dextran, and carboxyC₁₋₁₀alkyl-isomaltooligosaccharide; and

- a) wherein said cryoprotecting agent comprises at least 1% w/w of one or more of isomaltooligosaccharide and derivatives thereof having a weight average molecular weight (M_w) of between 300 and 1,650 Da based on the total weight of dextrin, dextran, isomaltooligosaccharide and derivatives thereof in said cryoprotecting agent,
or

- b) wherein said cryoprotectant has a weight average molecular weight (M_w) of between 300 and 9,500 Da,

for cryopreserving a sample, and wherein said sample is selected from the group consisting of mammalian organs, mammalian cells, and mammalian tissues.

In one aspect, there is provided a cryoprotecting agent comprising one or more cryoprotectants selected from the group consisting of dextrin, dextran, isomaltooligosaccharide, and derivatives thereof, which derivative is selected from the group consisting of hydrogenated isomaltooligosaccharide, hydrogenated dextran, hydrogenated dextrin, oxidised isomaltooligosaccharide, oxidised dextran, oxidised dextrin, DEAE-dextrin, DEAE-dextran, DEAE-isomaltooligosaccharides, carboxyC₁₋₁₀alkyl-dextrin, carboxyC₁₋₁₀alkyl-dextran, and carboxyC₁₋₁₀alkyl-isomaltooligosaccharide; and

- a) wherein said cryoprotecting agent comprises at least 1% w/w of one or more of isomaltooligosaccharide and derivatives thereof having a weight average molecular weight (M_w) of between 300 and 1,650 Da based on the total weight of dextrin, dextran, isomaltooligosaccharide and derivatives thereof in said cryoprotecting agent, or
- b) wherein said cryoprotectant has a weight average molecular weight (M_w) of between 300 and 9,500 Da.

In one aspect, there is provided a cryoprotecting agent comprising a cryoprotectant selected from the group consisting of isomaltooligosaccharide and derivatives thereof which derivative is selected from the group consisting of hydrogenated isomaltooligosaccharide, oxidised isomaltooligosaccharide, DEAE-isomaltooligosaccharides, carboxyC₁₋₁₀alkyl oxidised isomaltooligosaccharide and carboxyC₁₋₁₀alkyl-isomaltooligosaccharide, having a weight average molecular weight (M_w) of between 300 and 1,650 Da.

In one aspect, there is provided cryopreservation composition comprising a cryoprotecting agent as defined in any one of claims 1-12, and which cryopreservation composition further comprises a sample to be cryopreserved, wherein said sample is selected from the group consisting of mammalian organs, mammalian cells, and mammalian tissues.

So, in a first aspect-embodiment the present invention-disclosure relates to a cryoprotecting agent comprising one or more cryoprotectants selected from the group consisting of dextrin, dextran, isomaltooligosaccharide, and derivatives thereof, and

- a) wherein said cryoprotecting agent comprises at least 1% w/w of one or more of isomaltooligosaccharide and derivatives thereof having a weight average molecular weight (M_w) of between 300 and 1,650 Da based on the total weight of dextrin, dextran, isomaltooligosaccharide, and derivatives thereof, or
- b) wherein said cryoprotectant has a weight average molecular weight (M_w) of between 300 and 9,500 Da such as between 300 and 7,500 Da or,

- c) wherein said cryoprotecting agent comprises at least 1% w/w of one or more of isomaltooligosaccharide and derivatives thereof having a weight average molecular weight (M_w) of between 300 and 1,650 Da based on the total weight of dextrin, dextran, isomaltooligosaccharide and derivatives thereof in said cryoprotecting agent, and said cryoprotectant has a weight average molecular weight (M_w) of between 300 and 9,500 Da.

In a further ~~aspect~~ embodiment, the present ~~invention~~ disclosure relates to a cryoprotecting agent comprising a cryoprotectant selected from the group consisting of isomaltooligosaccharide and derivatives thereof having a weight average molecular weight (M_w) of between 300 and 1,650 Da, such as between 850 and 1,650 Da.

In a further ~~aspect~~ embodiment, the present ~~invention~~ disclosure relates to the use of a cryoprotectant as described herein for cryopreserving a sample, wherein said sample is selected from the group consisting of organs, cells and tissues.

In a further ~~aspect~~ embodiment, the present ~~invention~~ disclosure relates to a cryopreservation composition comprising a cryoprotecting agent as described herein, which cryopreservation composition further comprises a sample to be cryopreserved, wherein said sample is selected from the group consisting of organs, cells and tissues.

In a further ~~aspect~~ embodiment, the present ~~invention~~ disclosure relates to a cryopreserved composition comprising a cryoprotecting agent as described herein, which cryopreserved composition further comprises a sample which has been cryopreserved, wherein said sample is selected from the group consisting of organs, cells and tissues.

In a further ~~aspect~~ embodiment, the present ~~invention~~ disclosure relates to a method of cryopreserving a sample, comprising the steps of bringing a sample to be cryopreserved into contact with a cryoprotecting agent as described herein to obtain a cryopreservation composition and subsequently reducing the temperature of the cryopreservation composition to a cryopreservation temperature, wherein said sample is selected from the group consisting of organs, cells and tissues.

In a further ~~aspect~~ embodiment, the present ~~invention~~ disclosure relates to a method of cryopreserving a cryopreservation composition as described herein by bringing the composition to a cryopreservation temperature.

In a further ~~aspect~~ embodiment, the present ~~invention~~ disclosure relates to the use of a cryoprotecting agent as described herein for cryopreserving a sample, wherein said sample is selected from the group consisting of organs, cells and tissues.

In a further ~~aspect~~ embodiment, the present ~~invention~~ disclosure relates to the use of a cryoprotecting agent as described herein for cryopreserving a sample for transplantation.

In a further ~~aspect~~ embodiment, the present ~~invention~~ disclosure relates to the use of a cryopreservation composition as described herein for cryopreserving a sample by reducing the temperature of said composition to a cryopreservation temperature, wherein said sample is selected from the group consisting of organs, cells and tissue.

LEGENDS TO THE FIGURE

Fig. 1 shows survival of NHDFs after thawing and viability after 1. passage in respectively 1) 10% DMSO, 2) 8% isomaltooligosaccharide 1 (ISOM) and 2% DMSO, 3) 8% isomaltooligosaccharide 1 (ISOM) and 4) DMEM as described in Example 2.

Fig. 2 shows survival of NHDFs after thawing and viability after 1. passage in respectively 1) 10% DMSO, 2) 8% hydrogenated isomaltooligosaccharide 1 (H-ISOM) and 2% DMSO, 3) 8% hydrogenated isomaltooligosaccharide 1 (H-ISOM) and 4) DMEM as described in Example 2.

Fig. 3 shows survival of NHEKs after thawing and viability after 1. passage in respectively 1) 10% DMSO, 2) 8% isomaltooligosaccharide 1 (ISOM) and 2% DMSO, 3) 8% isomaltooligosaccharide 1 (ISOM) and 4) DMEM as described in Example 3.

Fig.4 shows survival of NHEKs after thawing in respectively 1) 10% DMSO, 2) 8% hydrogenated isomaltooligosaccharide 1 (H-ISOM) and 2% DMSO, 3) 8% hydrogenated isomaltooligosaccharide 1 (H-ISOM) and 4) DMEM as described in Example 3.

Fig. 5 shows survival of MSCs after thawing and viability after 1. passage in respectively 1) 10% DMSO, 2) 8% isomaltooligosaccharide 1 (ISOM) and 2% DMSO, 3) 8% hydrogenated isomaltooligosaccharide 1 (H-ISOM) and 2% DMSO, 4) 8% isomaltooligosaccharide 1 (ISOM), 5) 8% hydrogenated isomaltooligosaccharide 1 (H-ISOM) and 6) DMEM as described in Example 4.

Fig. 6 shows survival of Human iPS cells in PluriPro growth medium after thawing and cryopreservation in respectively Growth medium + 10% DMSO, Growth medium + 5%

CLAIMS - SEPT. 2014

1. Use of a cryoprotecting agent comprising one or more cryoprotectants selected from the group consisting of dextrin, dextran, isomaltooligosaccharide, and derivatives thereof
5 which derivative is selected from the group consisting of hydrogenated isomaltooligosaccharide, hydrogenated dextran, hydrogenated dextrin, oxidised isomaltooligosaccharide, oxidised dextran, oxidised dextrin, DEAE-dextrin, DEAE-dextran, DEAE-isomaltooligosaccharides, carboxyC₁₋₁₀alkyl-dextrin, carboxyC₁₋₁₀alkyl-dextran, and carboxyC₁₋₁₀alkyl-isomaltooligosaccharide; and
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- a) wherein said cryoprotecting agent comprises at least 1% w/w of one or more of isomaltooligosaccharide and derivatives thereof having a weight average molecular weight (M_w) of between 300 and 1,650 Da based on the total weight of dextrin, dextran, isomaltooligosaccharide and derivatives thereof in said cryoprotecting agent,
15 or
- b) wherein said cryoprotectant has a weight average molecular weight (M_w) of between 300 and 9,500 Da,
for cryopreserving a sample, and wherein said sample is selected from the group consisting
20 of mammalian organs, mammalian cells, and mammalian tissues.
2. The use according to claim 1, wherein said cryoprotecting agent comprises at least 1% w/w of one or more of isomaltooligosaccharide and derivatives thereof having a weight average molecular weight (M_w) of between 300 and 1,650 Da based on the total weight of
25 dextrin, dextran, isomaltooligosaccharide and derivatives thereof in said cryoprotecting agent.
3. The use according to any one of claims 1-2, wherein said cryoprotectant has a weight average molecular weight (M_w) of between 300 and 9,500 Da.
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4. The use according to claim 3, wherein said cryoprotectant has a weight average molecular weight (M_w) of between 300 and 7,500 Da.
5. The use according to any one of claims 1-4, wherein said cryoprotectant is selected
35 from the group consisting of isomaltooligosaccharide and derivatives thereof and has a weight average molecular weight (M_w) of between 850 and 1,650 Da.

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6. The use according to any one of claims 1-5, wherein said cryoprotectant has a polydispersity of ≥ 1 and ≤ 5 .
7. The use according to any one of claims 1-6, wherein said derivative is selected from the group consisting of hydrogenated isomaltooligosaccharide, hydrogenated dextran, hydrogenated dextrin, oxidised isomaltooligosaccharide, oxidised dextran, and oxidised dextrin, .
8. The use according to any one of claims 1-7, wherein said isomaltooligosaccharide derivative is selected from the group consisting of hydrogenated isomaltooligosaccharide and oxidised isomaltooligosaccharide.
9. The use according to any one of claims 1-8, wherein said cryoprotectant is isomaltooligosaccharide having a weight average molecular weight (M_w) of between 850 and 1,150 Da.
10. The use according to any one of claims 1-9, wherein said cryoprotectant is hydrogenated isomaltooligosaccharide having a weight average molecular weight (M_w) of between 850 and 1,150 Da.
11. The use according to any one of claims 9-10, wherein the weight fraction of isomaltooligosaccharides having less than 3 glucose units is less than 15% w/w.
12. The use according to any one of claims 9-11, wherein the weight fraction of isomaltooligosaccharides having more than 9 glucose units is less than 20 % w/w.
13. The use according to any one of claims 1-12, wherein said isomaltooligosaccharides and derivatives thereof are dextran-based.
14. The use according to any one of claims 1-13, wherein said sample is for transplantation.
15. The use according to any one of claims 1-14, wherein said agent is in the form of a powder or in the form of a solution.
16. The use according to any one of claims 1-15, wherein said agent comprises from 30% to 70% w/w of said cryoprotectant.

17. A cryoprotecting agent comprising one or more cryoprotectants selected from the group consisting of dextrin, dextran, isomaltooligosaccharide, and derivatives thereof, which derivative is selected from the group consisting of hydrogenated isomaltooligosaccharide, hydrogenated dextran, hydrogenated dextrin, oxidised isomaltooligosaccharide, oxidised dextran, oxidised dextrin, DEAE-dextrin, DEAE-dextran, DEAE-isomaltooligosaccharides, carboxyC₁₋₁₀alkyl-dextrin, carboxyC₁₋₁₀alkyl-dextran, and carboxyC₁₋₁₀alkyl-isomaltooligosaccharide; and
- 5
- a) wherein said cryoprotecting agent comprises at least 1% w/w of one or more of isomaltooligosaccharide and derivatives thereof having a weight average molecular weight (M_w) of between 300 and 1,650 Da based on the total weight of dextrin, dextran, isomaltooligosaccharide and derivatives thereof in said cryoprotecting agent, or
- 10
- b) wherein said cryoprotectant has a weight average molecular weight (M_w) of between 300 and 9,500 Da.
- 15
18. The cryoprotecting agent according to claim 17, wherein said cryoprotecting agent comprises at least 1% w/w of one or more of isomaltooligosaccharide and derivatives thereof having a weight average molecular weight (M_w) of between 300 and 1,650 Da based on the total weight of dextrin, dextran, isomaltooligosaccharide and derivatives thereof in said cryoprotecting agent.
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19. The cryoprotecting agent according to any one of claims 17-18, wherein said cryoprotectant has a weight average molecular weight (M_w) of between 300 and 9,500 Da.
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20. A cryoprotecting agent comprising a cryoprotectant selected from the group consisting of isomaltooligosaccharide and derivatives thereof which derivative is selected from the group consisting of hydrogenated isomaltooligosaccharide, oxidised isomaltooligosaccharide, DEAE-isomaltooligosaccharides, and carboxyC₁₋₁₀alkyl-isomaltooligosaccharide, having a weight average molecular weight (M_w) of between 300 and 1,650 Da.
- 30
21. The cryoprotecting agent according to any one of claims 17-20, wherein the cryoprotectant is as further defined in any one of claims 1-13.
- 35
22. The cryoprotecting agent according to any one of claims 17-21, which is as further defined in any one of claims 1-13 and 15-16.

23. A cryopreservation composition comprising a cryoprotecting agent as defined in any one of claims 1-22, and which cryopreservation composition further comprises a sample to be cryopreserved, wherein said sample is selected from the group consisting of mammalian organs, mammalian cells, and mammalian tissues.

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24. The cryopreservation composition according to claim 23, comprising said cryoprotectant in an amount from 1 to 50 % w/w.

10 25. The cryopreservation composition according to any one of claims 23-24, wherein said composition further comprises DMSO in an amount of less than 8% w/w, such as less than 5 % w/w, such as less than 4 % w/w.

15 26. A method of cryopreserving a sample, comprising the steps of bringing a sample to be cryopreserved into contact with a cryoprotecting agent as defined in any one of claims 1-22 to obtain a cryopreservation composition and subsequently reducing the temperature of the cryopreservation composition to a cryopreservation temperature, wherein said sample is selected from the group consisting of mammalian organs, mammalian cells, and mammalian tissues.

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27. The method according to claim 26, wherein said sample is for transplantation.

CLAIMS

1. Use of a cryoprotecting agent comprising one or more cryoprotectants selected from the group consisting of dextrin, dextran, isomaltooligosaccharide, and derivatives thereof
 5 which derivative is selected from the group consisting of hydrogenated isomaltooligosaccharide, hydrogenated dextran, hydrogenated dextrin, oxidised isomaltooligosaccharide, oxidised dextran, oxidised dextrin, DEAE-dextrin, DEAE-dextran, DEAE-isomaltooligosaccharides, carboxyC₁₋₁₀alkyl-dextrin, carboxyC₁₋₁₀alkyl-dextran, and carboxyC₁₋₁₀alkyl-isomaltooligosaccharide; and
 10
 - a) wherein said cryoprotecting agent comprises at least 1% w/w of one or more of isomaltooligosaccharide and derivatives thereof having a weight average molecular weight (M_w) of between 300 and 1,650 Da based on the total weight of dextrin, dextran, isomaltooligosaccharide and derivatives thereof in said cryoprotecting agent,
 15 or
 - b) wherein said cryoprotectant has a weight average molecular weight (M_w) of between 300 and 9,500 Da,
 for cryopreserving a sample, and wherein said sample is selected from the group consisting
 20 of mammalian organs, mammalian cells, and mammalian tissues.
2. The use according to claim 1, wherein said cryoprotecting agent comprises at least 1% w/w of one or more of isomaltooligosaccharide and derivatives thereof having a weight average molecular weight (M_w) of between 300 and 1,650 Da based on the total weight of
 25 dextrin, dextran, isomaltooligosaccharide and derivatives thereof in said cryoprotecting agent.
3. The use according to any one of claims 1-2, wherein said cryoprotectant has a weight average molecular weight (M_w) of between 500 and 3,500 Da.
 30
4. The use according to any one of claims 1-3, wherein said cryoprotectant is selected from the group consisting of isomaltooligosaccharide and derivatives thereof and has a weight average molecular weight (M_w) of between 850 and 1,650 Da.
 35
5. The use according to any one of claims 1-4, wherein said cryoprotectant has a polydispersity of ≥ 1 and ≤ 5 .

6. The use according to any one of claims 1-5, wherein said cryoprotectant is isomaltooligosaccharide having a weight average molecular weight (M_w) of between 850 and 1,150 Da.
- 5 7. The use according to any one of claims 1-6, wherein said cryoprotectant is hydrogenated isomaltooligosaccharide having a weight average molecular weight (M_w) of between 850 and 1,150 Da.
8. The use according to any one of claims 6-7, wherein the weight fraction of
10 isomaltooligosaccharides having less than 3 glucose units is less than 15% w/w and/or

wherein the weight fraction of isomaltooligosaccharides having more than 9 glucose units is less than 20 % w/w.
- 15 9. The use according to any one of claims 1-8, wherein said isomaltooligosaccharides and derivatives thereof are dextran-based.
10. The use according to any one of claims 1-9, wherein said agent is in the form of a powder or in the form of a solution.
- 20 11. The use according to any one of claims 1-10, wherein said agent comprises from 30% to 70% w/w of said cryoprotectant.
12. The use according to any one of claims 1-11, wherein said agent comprises at least
25 one additional cryoprotectant selected from the group consisting of acetamide, agarose, alginate, 1-analine, albumin, ammonium acetate, butanediol, chondroitin sulfate, chloroform, choline, diethylene glycol, dimethyl acetamide, dimethyl formamide, dimethyl sulfoxide (DMSO), erythritol, ethanol, ethylene glycol, formamide, glucose, glycerol, a-
glycerophosphate, glycerol monoacetate, glycine, hydroxyethyl starch, inositol, lactose,
30 magnesium chloride, magnesium sulfate, maltose, mannitol, mannose, methanol, methyl acetamide, methylformamide, methyl ureas, phenol, pluronic polyols, polyethylene glycol, polyvinylpyrrolidone, proline, propylene glycol, pyridine N-oxide, ribose, serine, sodium bromide, sodium chloride, sodium iodide, sodium nitrate, sodium sulfate, sorbitol, sucrose, trehalose, triethylene glycol, trimethylamine acetate, urea, valine and xylosen.

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13. The use according to any one of claims 1-12, wherein said sample is cells selected from the group consisting of hematopoietic stem cells, mesenchymal stem cells, embryonic stem cells and IPS cells.

5 14. A cryoprotecting agent comprising one or more cryoprotectants selected from the group consisting of dextrin, dextran, isomaltooligosaccharide, and derivatives thereof, which derivative is selected from the group consisting of hydrogenated isomaltooligosaccharide, hydrogenated dextran, hydrogenated dextrin, oxidised isomaltooligosaccharide, oxidised dextran, oxidised dextrin, DEAE-dextrin, DEAE-dextran, DEAE-isomaltooligosaccharides,
10 carboxyC₁₋₁₀alkyl-dextrin, carboxyC₁₋₁₀alkyl-dextran, and carboxyC₁₋₁₀alkyl-isomaltooligosaccharide; and

a) wherein said cryoprotecting agent comprises at least 1% w/w of one or more of isomaltooligosaccharide and derivatives thereof having a weight average molecular weight (M_w) of between 300 and 1,650 Da based on the total weight of dextrin, dextran, isomaltooligosaccharide and derivatives thereof in said cryoprotecting agent,
15 or

b) wherein said cryoprotectant has a weight average molecular weight (M_w) of between
20 300 and 9,500 Da.

15. A cryoprotecting agent comprising a cryoprotectant selected from the group consisting of isomaltooligosaccharide and derivatives thereof which derivative is selected from the group consisting of hydrogenated isomaltooligosaccharide, oxidised isomaltooligosaccharide, DEAE-isomaltooligosaccharides, carboxyC₁₋₁₀alkyl oxidised isomaltooligosaccharide and carboxyC₁₋₁₀alkyl-isomaltooligosaccharide, having a weight average molecular weight (M_w) of between
25 300 and 1,650 Da.

16. The cryoprotecting agent according to any one of claims 14-15, wherein the
30 cryoprotectant is as further defined in any one of claims 1-12.

17. A cryopreservation composition comprising a cryoprotecting agent as defined in any one of claims 1-12, and which cryopreservation composition further comprises a sample to be cryopreserved, wherein said sample is selected from the group consisting of mammalian
35 organs, mammalian cells, and mammalian tissues.

18. The cryopreservation composition according claim 17, wherein said sample is cells selected from the group consisting of hematopoietic stem cells, mesenchymal stem cells, embryonic stem cells and IPS cells.
- 5 19. The cryopreservation composition according to any one of claims 17-18, comprising said cryoprotectant in an amount from 1 to 50 % w/w.
20. A method of cryopreserving a sample, comprising the steps of bringing a sample to be cryopreserved into contact with a cryoprotecting agent as defined in any one of claims 1-13
10 to obtain a cryopreservation composition and subsequently reducing the temperature of the cryopreservation composition to a cryopreservation temperature, wherein said sample is selected from the group consisting of mammalian organs, mammalian cells, and mammalian tissues.
- 15 21. The method according to claim 20, wherein said sample is cells selected from the group consisting of hematopoietic stem cells, mesenchymal stem cells, embryonic stem cells and IPS cells.