

What is claimed is:

1. A cell culture medium comprising Advanced DMEM/F12, a glutamine source at a concentration from 1 to 5 mM, additional source of ascorbic acid, wherein the concentration
5 of ascorbic acid in the culture medium is from 0.1 to 0.8 mM, a member of the FGF superfamily at a concentration from 25 to 75 µg/L, a member of the TGF-beta superfamily of ligands selected from the group consisting of TGF-β1, TGF-β2 and TGF-β3 at a concentration from 0.75 to 2.25 µg/L, wherein the medium allows in vitro passaging of undifferentiated human pluripotent stem cells under serum-free, feeder cell-free conditions
10 for at least 50 passages, and wherein the undifferentiated cells express Oct4, Sox2 and Nanog, and maintain karyotypic stability for the at least 50 passages.
2. The cell culture medium of claim 1, wherein the culture medium does not contain one or more of gamma amino butyric acid (GABA), pipecolic acid (PA), and lithium (Li).
15
3. The cell culture medium of claim 1, wherein the culture medium does not contain one or more of 2-mercaptoethanol, pluronic F-68, and Tween 80.
4. The cell culture medium of claim 1, wherein the concentration of ascorbic acid in the
20 medium is from 0.1 mM to 0.6 mM.
5. The cell culture medium of claim 4, wherein the concentration of ascorbic acid is from 0.15 to 0.30 mM.
- 25 6. The cell culture medium of claim 1, wherein the member of the FGF superfamily is FGF1 or FGF2.
7. The cell culture medium of claim 1, wherein the TFG-beta superfamily of ligands is TGF-β1.
30
8. The cell culture medium of claim 1, wherein the glutamine is L-alanyl-L-glutamine.

9. The cell culture medium of claim 1, wherein the ascorbic acid is from 0.1 to 0.8 mM, the member of the FGF superfamily is from 25 to 200 µg/L, the TGF-β superfamily ligand is from 0.5 to 5 µg/L, and glutamine is from 1 to 5 mM.
- 5 10. The cell culture medium of claim 9, wherein the ascorbic acid is about 0.25 mM, FGF-2 is about 50 µg/L, TGF-beta superfamily ligand is about 1.5 µg/L, and glutamine is about 2.5 mM.
11. The cell culture medium of claim 1, wherein the albumin in the Advanced
- 10 DMEM/F12 is bovine serum albumin, human serum albumin, or recombinant human or bovine albumin.
12. A cell culture medium comprising the following components

Component	mg/L
L-alanyl glutamine	543
Ascorbic acid phosphate	63.5
FGF2	0.05
TGFβ1	0.0015
Glycine	18.75
L-Alanine	4.45
L-Arginine hydrochloride	147.5
L-Asparagine-H ₂ O	7.5
L-Aspartic acid	6.65
L-Cysteine hydrochloride-H ₂ O	17.56
L-Cystine 2HCl	31.29
L-Glutamic Acid	7.35
L-Histidine hydrochloride-H ₂ O	31.48
L-Isoleucine	54.47
L-Leucine	59.05
L-Lysine hydrochloride	91.25
L-Methionine	17.24
L-Phenylalanine	35.48
L-Proline	17.25
L-Serine	26.25
L-Threonine	53.45

L-Tryptophan	9.02
L-Tyrosine disodium salt dihydrate	55.79
L-Valine	52.85
Biotin	0.0035
Choline chloride	8.98
D-Calcium pantothenate	2.24
Folic Acid	2.65
Niacinamide	2.02
Pyridoxine hydrochloride	2
Riboflavin	0.219
Thiamine hydrochloride	2.17
Vitamin B12	0.68
i-Inositol	12.6
Calcium Chloride (CaCl ₂) (anhyd,)	116.6
Cupric sulfate (CuSO ₄ -5H ₂ O)	0.0013
Ferric Nitrate (Fe(NO ₃) ₃ -9H ₂ O)	0.05
Ferric sulfate (FeSO ₄ -7H ₂ O)	0.417
Magnesium Chloride (anhydrous)	28.64
Magnesium Sulfate (MgSO ₄) (anhyd,)	48.84
Potassium Chloride (KCl)	311.8
Sodium Bicarbonate (NaHCO ₃)	2438
Sodium Chloride (NaCl)	6995.5
Sodium Phosphate dibasic (Na ₂ HPO ₄) anhydrous	71.02
Sodium Phosphate monobasic (NaH ₂ PO ₄ -H ₂ O)	62.5
Zinc sulfate (ZnSO ₄ -7H ₂ O)	0.864
Lipid rich albumin	400
Transferrin	7.5
Insulin	10
Glutathione, monosodium	1
Ammonium Metavanadate	3.00E-04
Manganous Chloride	5.00E-05
Sodium Selenite	0.005
D-Glucose (Dextrose)	3151
Ethanolamine	1.9
Hypoxanthine Na	2.39
Linoleic Acid	0.042
Lipoic Acid	0.105

Phenol Red	8.1
Putrescine 2HCl	0.081
Sodium Pyruvate	110
Thymidine	0.365

present at the concentrations indicated $\pm$ 10%.

13. The cell culture medium of claim 12, wherein the components are present at the
5 concentrations indicated.

14. A method of culturing and maintaining human pluripotent stem cells comprising
growing cells in the culture medium of claim 1, wherein the cell culture medium is serum-
free.
10

15. The method of claim 14, wherein the cells are not exposed to feeder cells or
conditioned medium therefrom.

16. The method of claim 14, wherein the cells are maintained in culture for at least 30
15 passages, and optionally express pluripotency markers at least after 30 passages.

17. The method of claim 14, wherein the cells are grown on a substrate.

18. The method of claim 17, wherein the substrate is vitronectin, laminin or matrigel.
20

19. The method of claim 16, wherein the pluripotency markers are Nanog, Oct4 and/or
Sox2.

20. The method of claim 14, wherein the cells are human embryonic stem cells or human
25 induced pluripotent stem cells.

21. A population of cells obtained by the method of claim 14.

22. A kit for preparing a cell culture medium comprising
30 i) Advanced DMEM/F12;

- ii) a mixture comprising ascorbic acid phosphate, L-alanyl-L-glutamine, a member of the FGF superfamily, and a member of the TGF- β superfamily of ligands selected from the group consisting of TGF β 1, TGF β 2 and TGF β 3, wherein the components are provided such that the concentration of the TGF- β superfamily of ligands will be 0.75 to 2.25 μ g/L, the concentration of ascorbic acid will be 0.1 to 0.8 mM, the concentration of the FGF superfamily will be 25 to 75 μ g/L, and the concentration of L-alanyl-L-glutamine will be 1 to 5 mM; and
- iii) instructions for preparing the cell culture medium.
23. The kit of claim 22, wherein the albumin is xeno-free.
24. The kit of claim 22, wherein the member of the FGF superfamily is FGF1 or FGF2, and the member of the TGF- β superfamily of ligands is TGF- β 1.
25. The cell culture medium of claim 1, wherein the proliferation rate of the human pluripotent stem cells (hPSCs) is at least 1.5 times the proliferation rate of the hPSCs cultured in mTeSR1 under similar culture conditions.
26. The cell culture medium of claim 25, wherein the proliferation rate of the hPSCs and the expression of pluripotency marker Nanog is at least twice that of hPSCs cultured in mTeSR1 under similar culture conditions.