Laminin-511 and recombinant vitronectin supplementation enables human pluripotent stem cell culture and differentiation on conventional tissue culture polystyrene surface in xeno-free conditions

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Supplementary Information

MaterialsAbbreviationCatalog No.CompanyMatrigelMatrigel#356230Corning (Corning, NY, USA)Recombinant vitronectinrVTA14700Thermo Fisher Scientific Inc. (Waltham, MA, USA)Laminin 511L-511B2011Nippi Inc. (Tokyo, Japan)Laminin 521L-521BLA-LNS21-02Veritas (Tokyo, Japan)Cell culture dishesCell culture dishesCell culture dishes6-well polystyrene plateTCP#353046Sigma-Aldrich (St. Louis, MO, USA)N-bydrosytoscinimideNHS13062Sigma-Aldrich (St. Louis, MO, USA)N-bydrosytoscinimideNHS3450Sigma-Aldrich (St. Louis, MO, USA)N-bydrosytoscinimideNHS13062Sigma-Aldrich (St. Louis, MO, USA)N-bydrosytoscinimideNHS13062Sigma-Aldrich (St. Louis, MO, USA)N-bydrosytoscinimideNHS13062Sigma-Aldrich (St. Louis, MO, USA)N-bydrosytoscinimideNHS13062Sigma-Aldrich (St. Louis, MO, USA)N-bydrosytoscinimideNHS13062Thermo Fisher Scientific Inc. (Waltham, MA, USA)Essential 8 mediumEssential 8A1510401Thermo Fisher Scientific Inc. (Waltham, MA, USA)Recolar 14 mediumEssential 8A1510401Thermo Fisher Scientific Inc. (Waltham, MA, USA)Recolar 24BochstPA-3014Lonza (Basel, Sviizzraha)P32705 Supplement, minusB277T504044Thermo Fisher Scientific Inc. (Waltham, MA, USA)B-27705 Supplement, minusB277Santa Cruz Biotechnology (Dallas, TX, USA) <th>Supplementary rable</th> <th>Waterials used in t</th> <th>ins study.</th> <th></th>	Supplementary rable	Waterials used in t	ins study.		
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Mouse IgG1 Isotype antibody Alexa Fluor 488 goat anti- mouse IgGIsotype-control Alexa Fluor 488 goat anti- 	Anti-cTnT antibody	Anti-cTnT antibody	MA5-12960	Thermo Fisher Scientific Inc. (Waltham, MA, USA)	
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Supplementary Table 1 Materials used in this study.

A. Pluripotent protein expression



B. Differentiated protein expression



Supplementary Fig. 1 Pluripotent protein and differentiation protein expression of hiPSCs (H-M5) after long-term cultivation for ten passages on untreated TCP dishes in the supplemented method using Mix-0.2 conditionS in xeno-free culture condition. (A) Pluripotency protein expression of Oct3/4 (i, green), Nanog (ii, red), Sox2 (v, green), and SSEA-4 (vi, red) in hiPSCs (H-M5) evaluated by immunostaining method, with nuclear staining by Hoechst 33342 (blue, iii, vii). The photos (iv) and (viii) were merged from the photos of (i) – (iii) and (v) – (vii), respectively. Scale bar indicates 100 μ m. (B) Evaluation of the differentiation ability of hiPSCs (H-M5) *in vitro* after long-term cultivation for ten passages on untreated TCP dishes in the supplemented method using Mix-0.2 condition in xeno-free culture condition. Morphology of EB cells differentiated from hPSCs (i). Expression of a mesodermal marker protein (ii, α -SMA, green), an ectodermal marker protein (v, GFAP, red) and an endodermal marker protein (vi, AFP, green) from EB cells evaluated by the immunostaining method, with nuclear staining from Hoechst 33342 (iii, vii, blue). The photos (iv) and (viii) were merged from the photos of (ii) – (vii), respectively. The scale bar indicates 100 μ m.

Α



Supplementary Fig. 2 Cardiac differentiation of hESCs (H9) after hESC culture for ten passages on untreated TCP dishes in the supplemented method using Mix-0.2 condition in xeno-free culture condition. (A) Timeline of the differentiation protocol for hESCs into cardiomyocytes. (B) The sequential morphological observation during cardiac induction of hESCs at day 0, 4, 12, and 17. The scale bar shows 100 μ m. (C) Immunostaining evaluation of hESC-derived cardiomyocytes. Expression of α -actinin (a and i, green), NKX2.5 (b, red), MLC2a (e, green), cTnT (f, red) and ML2Cv (j, red) on hESC-derived cardiomyocytes after 21 day of differentiation. DAPI (c, g, and k) was utilized for nuclei staining. The photos (d), (h) and (l) were created by merging (a) – (c), (e) – (g), and (i) – (k), respectively. The scale bar shows 100 μ m. (D) The expression of cardiac marker, cTnT on hESC-induced cardiomyocytes were evaluated using flow cytometry.