Wako innovación tecnológica para laboratorio Rafer

Life Science

Serum-free medium for undifferentiated cells maintenance

StemSure hPSC Medium Δ

StemSure hPSC Medium Δ is appropriate for maintaining human pluripotent stem cells (hPSCs), human ES cells (hESCs) and human iPS cells (hiPSCs), in a feeder-free, serum-free and animal-free environment. This medium does not contain animal derived component and albumin, so has not the difference between the lot, and stable culture is possible. Adding Y-27632 to this medium makes it possible to passage in a single cell.

StemSure hPSC Medium Δ does not contain bFGF. It is possible to use Matrigel®, iMatrix-511 or vitronectin as a coating reagent. It is possible to use hPSC Dissociation Solution, StemPro® Accutase, TrypLE™ Select or TrypLE™ Express.

- **For feeder-free culture**
- **Not contain animal derived components**
- **Low difference between the lot because albumin is not contained**
- **Quality control with human iPS cells**

Follows are simple protocol when you use 6-well plate. Please refer the manual attached to a product for the detail protocols.

Preparation of complete medium

1. Thaw StemSure hPSC Medium Δ at 2-10 degree for several hours or overnight.
2. Add 35-100 ng/ml basic fibloblast growth factor (bFGF)(final concentration) to the thawed StemSure hPSC Medium Δ, and gently mix well to prepare complete medium (sshPSC medium).

Preparation of Matrigel® coated plate

Transferring of hPSCs from the culture on feeder layers to the feeder-free culture

1. Aspirate the culture medium of a feeder-dependent cultured well.
2. Add 0.4 ml of CTK solution per well of 6-well plate.
3. Incubate at 37℃ with 5% CO_2 for 5 minutes.
4. Aspirate the CTK solution, and add 2 ml per well of the sshPSC medium and suspend the colonies in small clumps of about 100 cells by pipetting.
5. Transfer the cell suspension to a tube, and 6 ml of the sshPSC medium and gently mix by inverting the tube several times.
6. Spin at 1,000rpm at room temperature for 3 minutes.
7. Remove the supernatant, and add 2 ml per well of ROCKi+ medium※ and resuspend the pellet.
   ※ ROCKi+ medium：sshPSC medium containing 10 μmol/l Y-27632.
8. Transfer 2 ml of the cell suspension to the well coated by Matrigel®.
9. Incubate at 37℃ with 5%CO_2.
10. Replace the medium daily with 2 ml of sshPSC medium per well.
11. Passage the cells at 5-7 days.

Preparation of Matrigel® coated plate

Passaging in sshPSC medium

1. Aspirate the culture medium, and rinse the cells once with D-PBS(-).
2. Remove D-PBS(-), and add 1 ml of hPSC Dissotiation Solution, StemPro® Accutase, TrypLE™ Select or TrypLE™ Express per well.
3. Incubate at 37℃ with 5% CO_2 for 5 minutes.
4. Add 2 ml of the ROCKi+ medium, and suspend the colonies to single cells.
5. Transfer the cell suspension to a tube, and spin at 1,000 rpm at room temperature for 3 minutes.
6. Aspirate the supernatant and suspend the pellet with 2 ml of the ROCKi+ medium.
7. Count the viable cell numbers.
8. Add 2 ml of the ROCKi+ medium per well, and plate 1×10^5 cells per well Matrigel® coated, and plate 1×10^5 cells per well.
9. Incubate at 37℃ with 5%CO_2.
10. The next day (at day 1), replace the medium with 2 ml of the sshPSC medium per well, not the ROCKi+ medium.
11. Replace the medium daily with 2 ml of the sshPSC medium per well. The replacement of medium is unnecessary on day 2.
12. Passage the cells when cells grow to cover approximately 80-90% of the well, usually every 4-6 days.

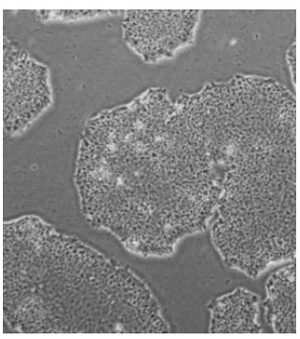
〔Cautions〕

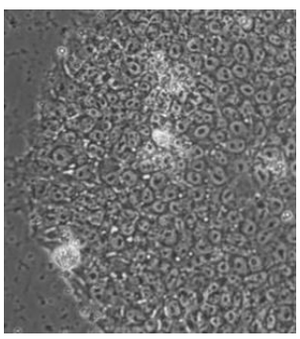
1. StemSure hPSC Medium Δ does not contain bFGF.
2. After thawing, store at 2-10℃ in the dark and use within a week.

Evaluation of colony formation, cell growth, maintenance of undifferentiated state and differentiation

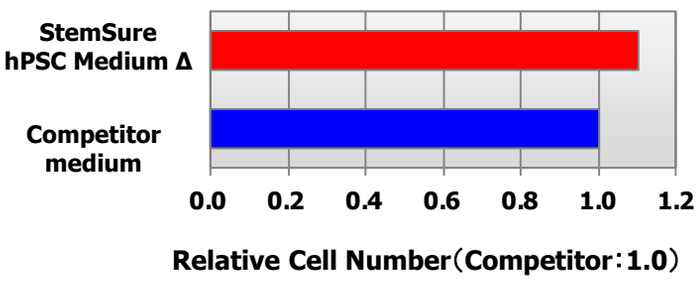
Culture hiPSCs (201B7 strain) with this StemSure hPSC Medium Δ, and evaluate colony formation, cell growth, maintenance of undifferentiated state and differentiation to three germ layer after formation of embryo body.

Colony formation

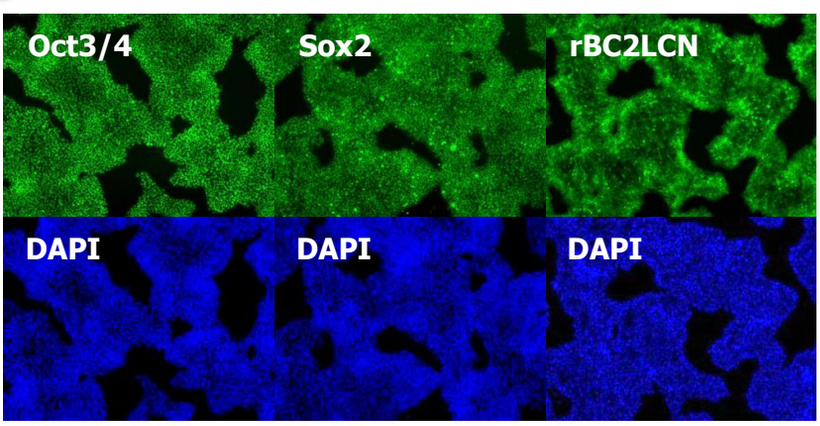




Cell Growth



Maintenance of undifferentiated state

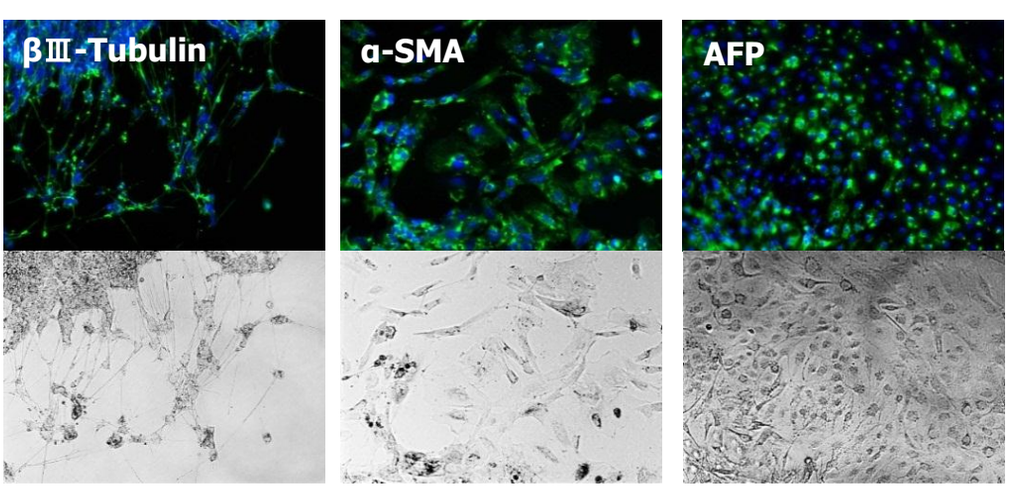


〔 Cell 〕
human iPS cells (201B7 strain)
〔 Culture Medium 〕
StemSure hPSC Medium Δ + 35ng/ml bFGF
〔 Coating Reagent 〕
Matrigel® hESC-Qualified Matrix
〔 Seeded Cell Number 〕
1×10^5 cells/well（6-well plate）
〔 Culture Days 〕
5 days

※ rBC2LCN is recombinant lectin which combines the sugar chain exists on the surface of hPSCs.

Differentiation to three germ layer

Culture hiPSCs (201B7 strain) in StemSure hPSC Medium Δ (passage 3), form embryo body, differentiate into three germ layer. Check the expression of differentiated marker (βⅢ-Tubulin, α-SMA, AFP).



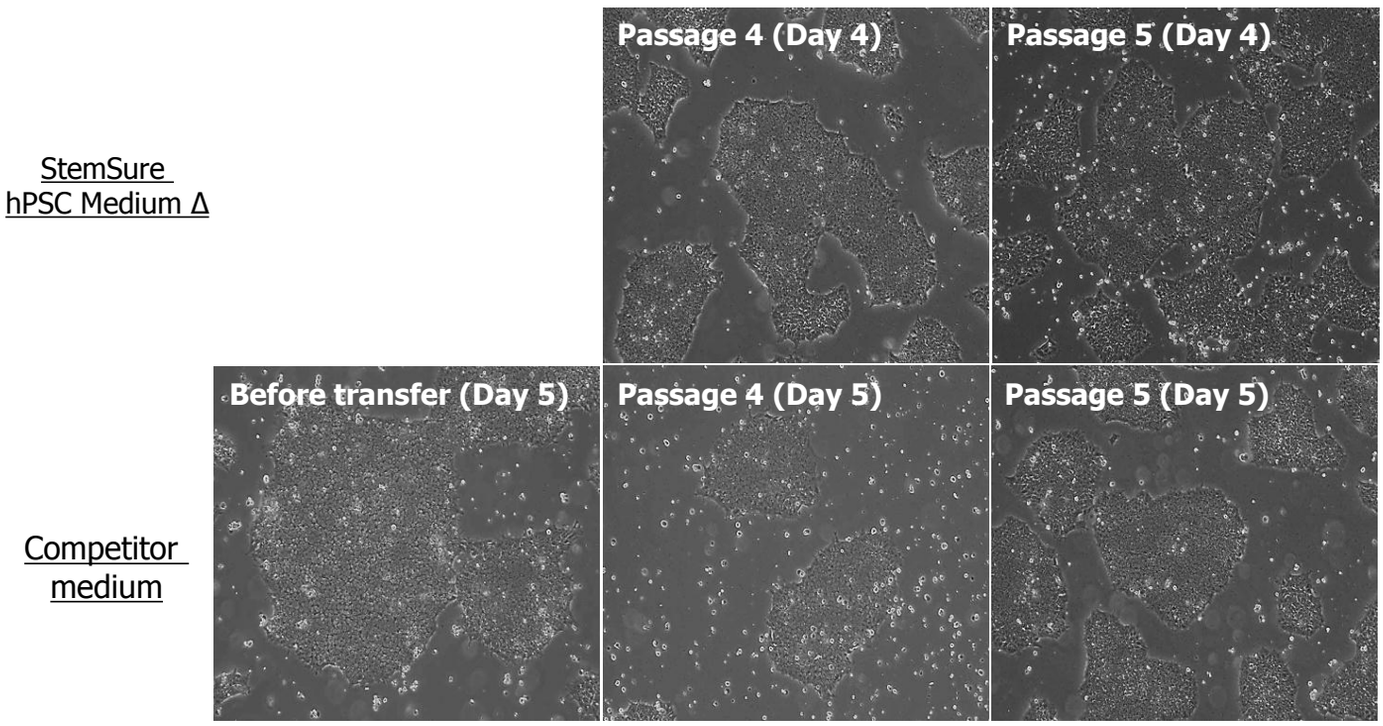
〔 Culture Medium of induction of differentiation 〕
StemSure D-MEM + 20% StemSure Serum Replacement + 2mmol/l L-Glutamine + 0.1mmol/l StemSure 2-Mercaptoetanol + 1 x Non-essential Amino Acids Solution

Culture of hiPSCs (201B7 strain)

Transferred hiPSCs (201B7 strain) which cultured in competitor medium (containing BSA, for feeder-free) to StemSure hPSC Medium Δ and culture passage. Show the colony formation, total population doubling level and maintenance of undifferentiated state below.

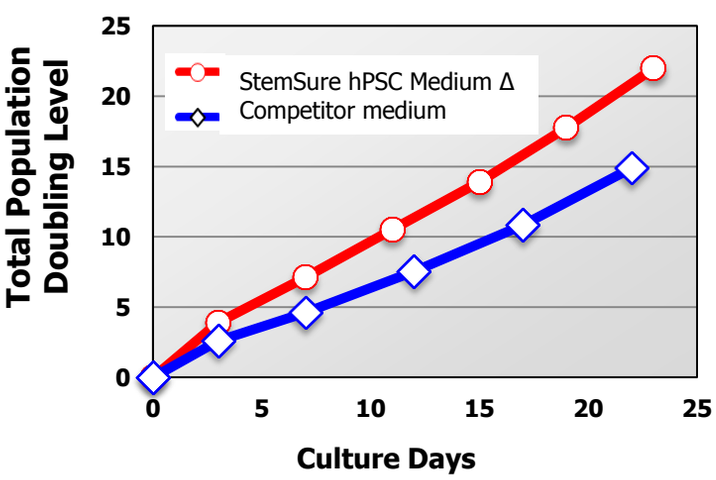
Colony formation

hiPSCs which were cultured in StemSure hPSC Medium Δ showed colony formation same as hiPSCs which were cultured in competitor medium, and there were not differentiated cells in medium.



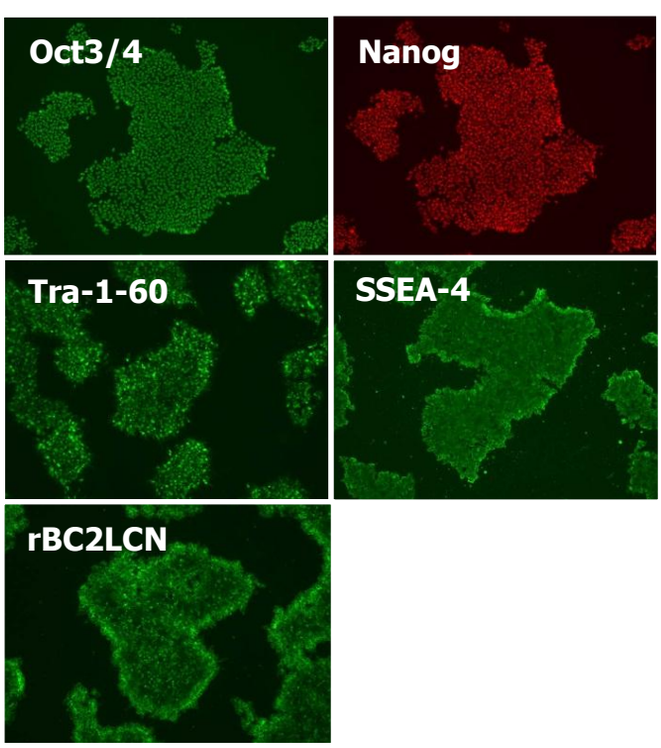
Total population doubling level

hiPSCs which were cultured in StemSure hPSC Medium Δ had better cell growth.



Maintenance of undifferentiated state

Cultured in StemSure hPSC Medium Δ (passage 5), and checked the expression of undifferentiated marker (Oct3/4, Nanog, Tra-1-60, SSEA-4, rBC2LCN).



〔 **Culture Medium** 〕
StemSure hPSC Medium Δ + 35ng/ml bFGF
〔 **Coating Reagent** 〕
Matrigel®
〔 **Seeded Cell Number** 〕
1×10^5 cells/well (6-well plate)

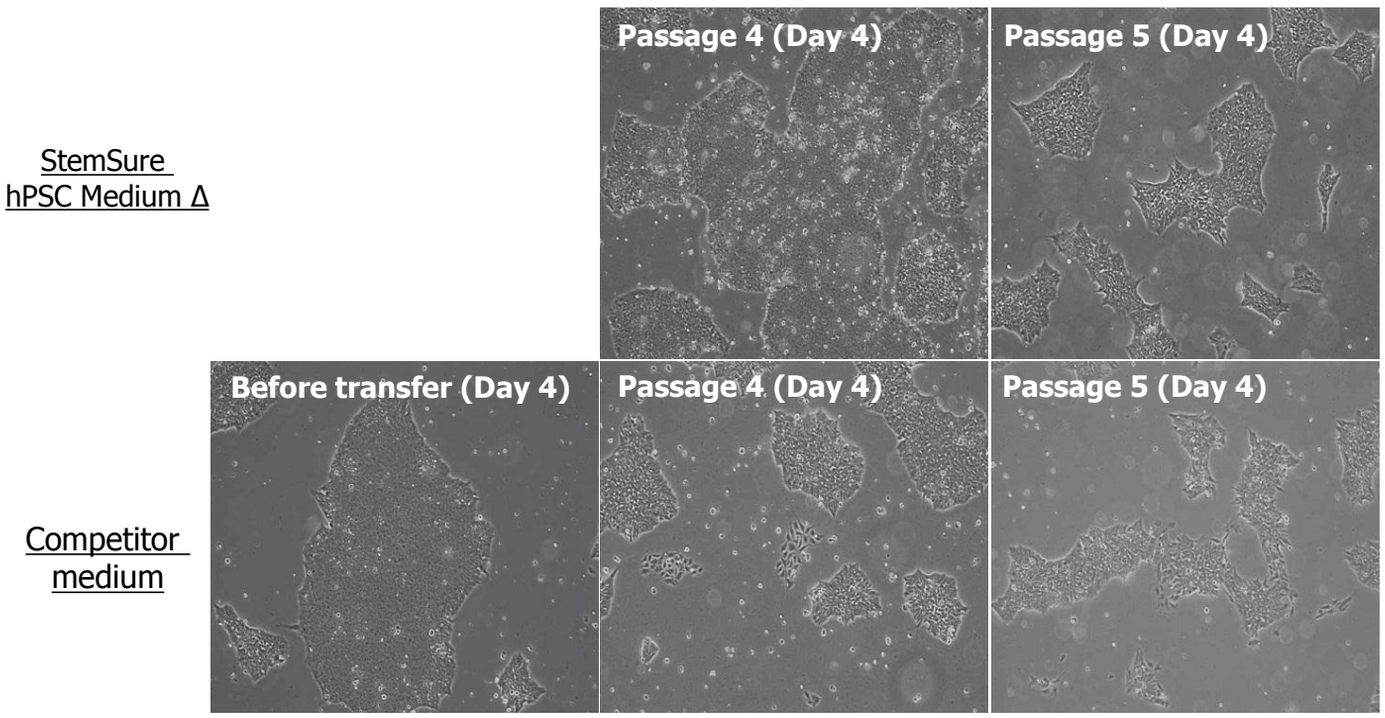
＜data from National Institute of Advanced Industrial Science and Technology, Dr. Onuma and Dr. Ito ＞

Culture of hESCs (WA01 strain)

Transferred hESCs (WA01 strain) which cultured in competitor medium (containing BSA, for feeder-free) to StemSure hPSC Medium Δ and culture passage. Show the colony formation, total population doubling level and maintenance of undifferentiated below.

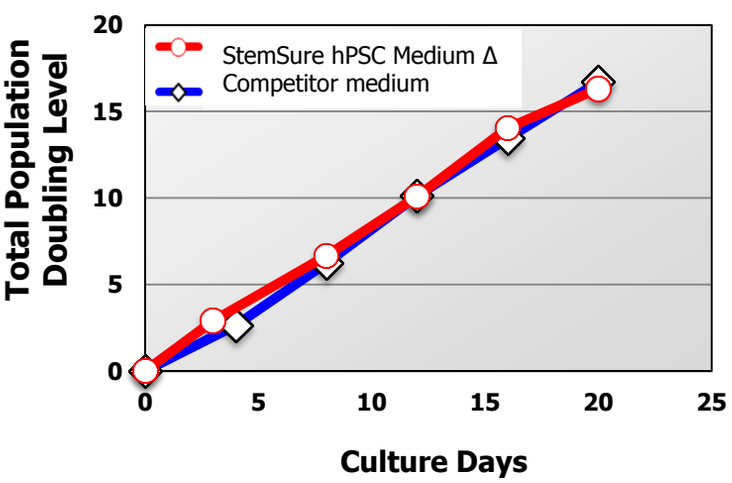
Colony formation

When hESCs were cultured in competitor medium, it partly appeared differentiated colony. But when hESCs were cultured in StemSure hPSC Medium Δ, it didn't appear differentiated colony.



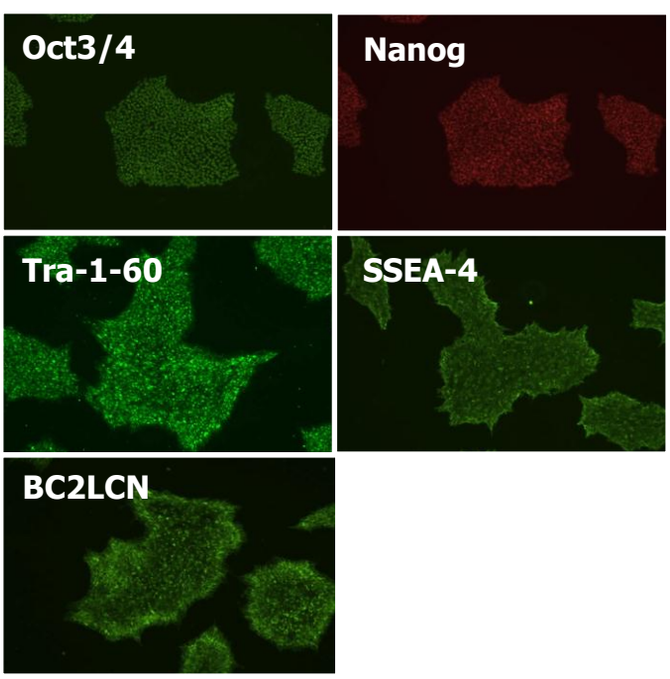
Total population doubling level

StemSure hPSC Medium Δ showed cell growth that was equal to competitor medium.



Maintenance of undifferentiated state

Cultured in StemSure hPSC Medium Δ (passage 5), and checked the expression of undifferentiated marker (Oct3/4, Nanog, Tra-1-60, SSEA-4, rBC2LCN).

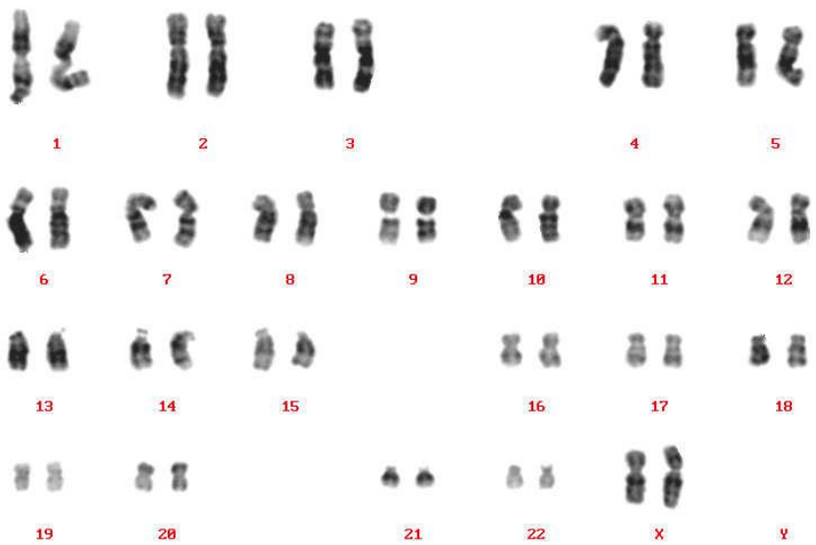


〔 **Culture Medium** 〕
StemSure hPSC Medium Δ + 35ng/ml bFGF
〔 **Coating Reagent** 〕
Matrigel®
〔 **Seeded Cell Number** 〕
1×10^5 cells/well (6-well plate)

＜data from National Institute of Advanced Industrial Science and Technology, Dr. Onuma and Dr. Ito ＞

Karyotype analysis

Analyzed karyotype of hiPSCs cultured in StemSure hPSC Medium Δ (passage 27), and confirmed that there was not chromosome abnormality.



< data from National Institute of National Center for Child Health and Development, Dr. Miura and Dr. Akutsu >

Code No.	Product Name	Grade	Package
197-17571 193-17573	StemSure hPSC Medium Δ	for Cell Culture	100mL 100mL×4

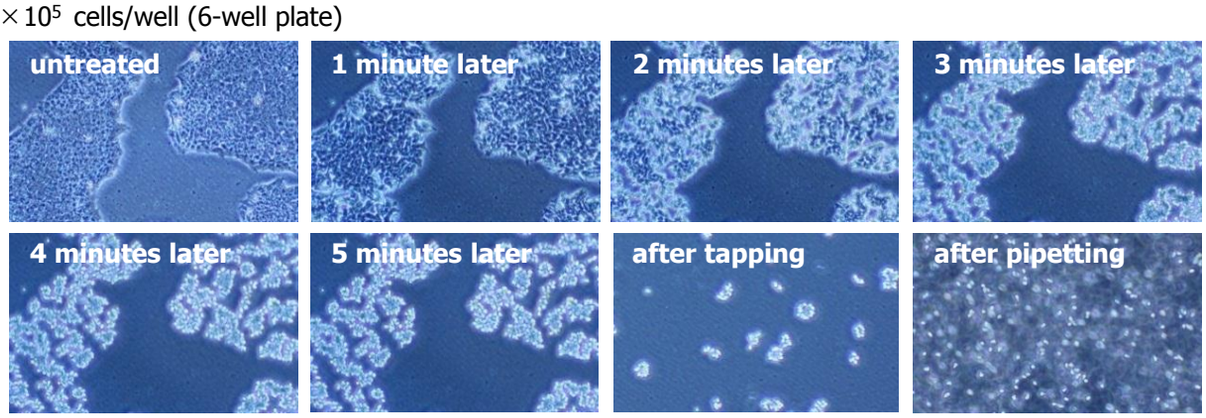
hPSC Dissociation Solution

hPSC Dissociation Solution is cell dispersion solution applicable for passage of hPSCs which are cultured under feeder-free condition. This product doesn't contain animal derived components and any enzymes such as trypsin and collagenase.

Add hPSC Dissociation Solution to culture medium of hiPSCs (201B7 strain) which were cultured in StemSure hPSC Medium Δ, and observed the dissociation state of hiPSCs. Addition 5 minutes later, tapped culture plate, dispersed into single cells by pipetting. And, after cultured using hPSC Dissociation Solution (passage 5), and check the expression of undifferentiated marker.

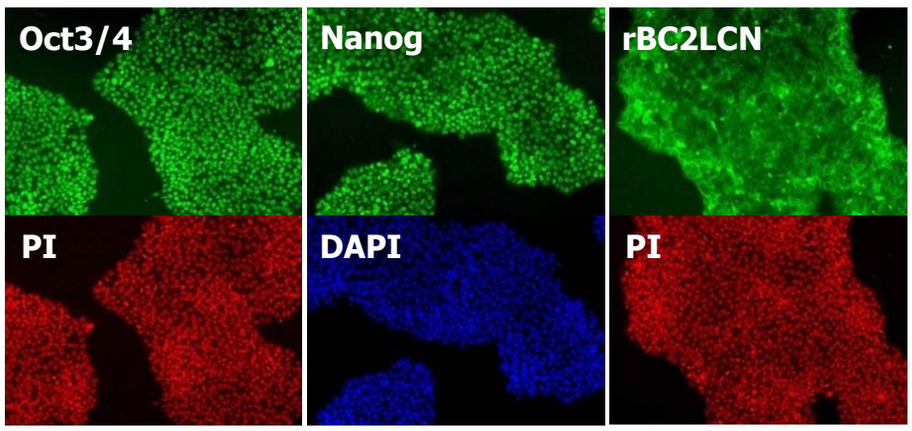
〔 Cell 〕
human iPS cells (201B7 strain)
〔 Culture Medium 〕
StemSure hPSC Medium Δ + 35ng/ml bFGF
〔 Coating Reagent 〕
Matrigel® hESC-Qualified Matrix
〔 Seeded Cell Number 〕
1×10^5 cells/well (6-well plate)

〔 Caution 〕
As it is different in adhesive property of a cell to a culture container depending on the cell kind and coating reagents, please treat with hPSC Dissociation Solution until cells are detached by tapping.



Maintenance of undifferentiated date

Checked the expression of undifferentiated marker (Nanog, Oct3/4, rBC2LCN).

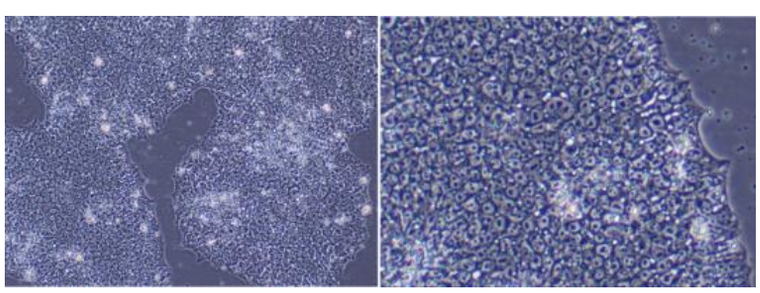


Code No.	Product Name	Grade	Package
160-27051	hPSC Dissociation Solution	for Cell Culture	100mL

recombinant Vitronectin

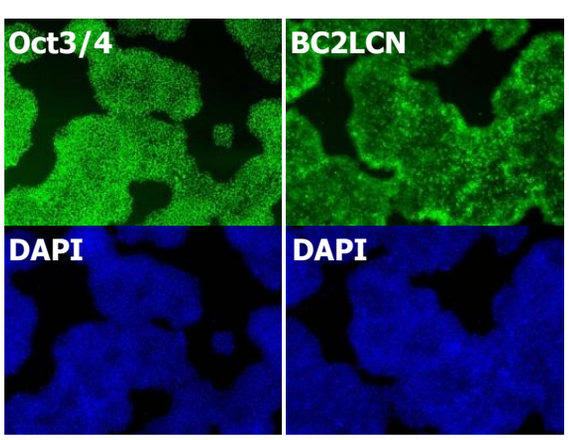
Vitronectin is the sugar protein exists in serum and extracellular matrix. This product is recombinant protein which is consisted of the 20-398 amino acids fragment except signal domain. This product is applicable for the culture of hiPSCs.

Colony formation



〔 **Product Outline** 〕
Assay：over 90% (SDS-PAGE)
Host：*E. coli*
Concentration：0.5mg/ml
Composition：20mM Tris-HCl, pH8.0
(contain NaCl, KCl, EDTA, Arginine, DTT and glycerol)

Maintenance of undifferetiated state



〔 **Cell** 〕
human iPS Cells (201B7 strain)
〔 **Culture Medium** 〕
StemSure hPSC Medium Δ + 32ng/ml bFGF
〔 **Coating Reagent** 〕
this product
〔 **Seeded Cell Number**〕
1×10^5 cells/well (6-well plate)
〔 **Paddage** 〕
Passage 6

Code No.	Poduct Name	Grade	Package
220-02041	Vitronectin(20-398aa), Human, recombinant, Solution	for Biochemistry	500μg

Related products

Code No.	Product Name	Grade	Package
064-05381 068-05384 060-05383	Fibroblast Growth Factor (basic), Human, recombinant, Animal-derived-free【bFGF/FGF2】	for Cellbiology	50μg 100μg 1mg
030-24021 036-24023 034-24024	CultureSure Y-27632 endotoxin tested, mycoplasma tested	for Cell Culture	1mg 5mg 25mg
257-00511 253-00513 251-00514 257-00516	Y-27632	for Cellbiology	1mg 5mg 25mg 1g
039-24591	10mmol/l CultureSure Y-27632 Solution	for Cell Culture	300μL
029-18061 025-18063	BC2LCN【AiLecS1】Lectin, recombinant, Solution BC2LCN has very high specificity to sugar chain which exists in cell surface of undifferentiated hPSCs.	Glycobiology	1mg 1mg×5
180-02991 186-02993	rBC2LCN-FITC【AiLecS1-FITC】 Excitation 495nm, Emission 520nm	for Cell Staining	100μL 100μL×5
186-03211 182-03213	rBC2LCN-547【AiLecS1-547】 labelled with yellow fluorescence dye, similar to Cy3 Excitation 551nm, Emission 565nm	for Cell Staining	100μL 100μL×5
185-03161 181-03163	rBC2LCN-635【AiLecS1-635】 labelled with red fluorescence dye, similar to Cy5 Excitation 634nm, Emission 654nm	for Cell Staining	100μL 100μL×5
197-16275	StemSure D-MEM（High Glucose）with Phenol Red and Sodium Pyruvate	for Cell Culture	500mL
197-16775	StemSure Serum Replacement【SSR】	for Cell Culture	500mL
198-15781	StemSure 10mmol/l 2-Mercaptoethanol Solution（×100）	for Cell Culture	100mL
195-16031	StemSure Freezing Medium Solution for cryopreservation applicable for hPSCs. Contain BSA and DMSO.	for Cell Culture	100mL
197-17831	StemSure hPSC Freezing Medium, AF Solution for cryopreservation applicable for hPSCs. Animal component free.	for Cell Culture	100mL

Listed products are intended for laboratory research use only, and not to be used for drug, food or human use. / Please visit our online catalog to search for other products from Wako; http://www.e-reagent.com / This leaflet may contain products that cannot be exported to your country due to regulations. / Bulk quote requests for some products are welcomed. Please contact us.