USER MANUAL

DEF-CS™ 100 - Defined Culture System for Maintenance of Human Induced Pluripotent Stem Cells

Contents

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General information

CATALOGUE #:	Y30020, DEF-CS™ 100:	1 bottle à 100 mL of DEF-CS™ Basal medium 1 vial à 800 µL of DEF-CS™ COAT-1 (for 100mL) 1 frozen vial à 300 µL of DEF-CS™ GF-1 (for 100mL) 1 frozen vial à 100 µL of DEF-CS™ GF-2 (for 100mL) 1 frozen vial à 40 µL of DEF-CS™ GF-3 (for 100mL)

DEF-CS™ is a complete system for efficient expansion and scale up manufacturing of human induced pluripotent stem cells (hiPSC) in a feeder free and defined environment.

The DEF-CS™ is shipped in a cooled box (DEF-CS™ Basal medium and COAT-1) and on dry ice (DEF-CS™ GF-1, GF-2, and GF-3) and should be handled according to “Storage and Handling of DEF-CS™ Components” upon arrival, see page 3.

All procedures described in the manual are optimised for DEF-hiPSC™ (ChiPSC4 and ChiPSC18). If you wish to use DEF-CS™ for other human induced pluripotent stem cells, please be aware that procedures and protocols may have to be adjusted.

We recommend that this product is handled only by persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good cell culture practice.

Additional Material Required

Product	Suggested Manufacturer	Catalogue number
PBS Dulbecco's with Ca^{2+} & Mg^{2+} (D-PBS +/+)	Gibco/Life Technologies	14040
PBS Dulbecco's w/o Ca^{2+} & Mg^{2+} (D-PBS -/-)	Gibco/Life Technologies	14190
TrypLE™ Select*	Gibco/Life Technologies	12563-029
Cell culture units	Becton-Dickinson Falcon	Tissue culture treated polystyrene surface

* other digestive enzymes can be used however this might need to be further optimised by the user

Other Equipment Needed

General cell culture equipment used in cell culture laboratory.

Product Quality

Takara Bio Europe AB recommends the use of media and reagents according to this manual. Takara Bio Europe AB cannot give technical feedback on customer cultures unless the below culture instructions have been followed.

Methods

NOTE! Always work under aseptic conditions.

Storage and Handling of DEF-CS™ Components

DEF-CS™ Basal medium
DEF-CS™ Basal medium is supplied in a cooled box. Store at +2-8˚C. Expiry date according to the label.

Preparation of culture medium by addition of DEF-CS™ GF-1, GF-2 and GF-3 to DEF-CS™ Basal medium should be made at day of use. The DEF-CS™ Basal medium should be warmed to +37±1°C before use. Discard any leftover warmed medium. The medium formulation contains penicillin and streptomycin.

DEF-CS™ GF-1, GF-2, and GF-3
DEF-CS™ GF-1, GF-2 and GF-3 are supplied frozen. Expiry dates can be found on the vials. Upon receipt, thaw provided vials and aliquot into appropriate volumes. Do not vortex. Store at ≤ -15°C according to expiry date on original vial. Thawed vials may be stored at +2-8˚C for up to 1 week. Do not re-freeze aliquots after thawing.

DEF-CS™ COAT-1
DEF-CS™ COAT-1 is supplied in a cooled box. Store at +2-8°C, see expiry date on the vial.

Storage and Handling of Frozen DEF-hiPSC™

The DEF-hiPSC™ are provided frozen in vials and are delivered on dry ice. The vials should be transferred to liquid N_2 when received or thawed according to procedure for thawing of frozen DEF-hiPSC™.

DEF-hiPSC™ are supplied from fully characterised cell banks and we recommend a maximum of 10 passage expansions from each vial to ensure consistent phenotype and genotype.

It is recommended that the cells are grown to a maximum confluence of 150-300k/cm^2.
DEF-hiPSC™ should be maintained in an incubator at +37±1˚C, 5 % CO_2 and > 90 % humidity.

Coating of Cell Culture Flasks

1. Dilute the required volume of DEF-CS™ COAT-1 in D-PBS (+/+) before use. Make a 1:20 dilution.
2. Mix the diluted DEF-CS™ COAT-1 solution gently and thoroughly by pipetting up and down.
3. Add the appropriate volume of diluted DEF-CS™ COAT-1 solution to the cell culture flasks (use 0.1 mL/cm^2), make sure the entire surface is covered.
4. Place the cell culture flasks for a minimum of 20 minutes in the incubator at +37±1°C or 0.5-3 h at room temperature (RT, +15-25°C).
5. Aspirate DEF-CS™ COAT-1 solution from cell culture flasks just before use.
6. The cell culture flasks are now coated and can be used for DEF-hiPSC™ culture.

Thawing of Frozen DEF-hiPSC™

Preparations
One vial of DEF-hiPSC™ should be thawed in one 12.5 cm^2 cell culture flask, in 5 mL of supplemented DEF-CS™ medium, or in 1 well of a 6 well plate, in 4 mL supplemented DEF-CS™ medium. Cell culture units should be coated as described above before use.
The appropriate volume of supplemented DEF-CS™ medium is prepared by adding DEF-CS™ GF-1 (dilute 1:333), GF-2 (dilute 1:1000) and GF-3 (dilute 1:1000) to DEF-CS™ Basal medium at the day of use. Discard any left-over warm medium.

NOTE! For your protection: Wear a protective face mask and protective gloves. Use forceps when handling a frozen vial. Never hold the vial in your hand as the cryo vial may explode due to rapid temperature changes.

Thawing cells
1. Transfer 9 mL of supplemented DEF-CS™ medium to a sterile centrifuge tube and warm to RT.
2. Using forceps, transfer the vial directly into a container with +37±1°C water. Thaw the vial by gently pushing it under the surface of the water. Do not submerge the cap of the vial in the water bath as this could contaminate the cells. Never hold the vial in your hand as the cryo vial may explode due to rapid temperature changes.
3. Allow the vial to thaw until the cell suspension can be poured out of the vial, with frozen parts of cell suspension still left in the vial.
4. Decontaminate the vial in appropriate disinfectant.
5. Pour the content of the vial into the sterile tube containing 9 mL supplemented DEF-CS™ medium (RT).
6. Use 1 mL supplemented DEF-CS™ medium, warmed to RT, to rinse the vial. Add to the cell suspension.
7. Centrifuge at 300g for 1 minute.
8. After centrifugation, aspirate the supernatant and gently resuspend the pellet in 4 or 5 mL supplemented DEF-CS™ medium (+37±1°C).
9. Pipette the cell suspension into the cell culture unit.
10. Ensure the cells and medium are evenly distributed across the surface of the cell culture unit and place the cell culture unit in the incubator.

Passage of DEF-hiPSC™

Preparations
Cell culture flasks should be coated as described above before use.
The appropriate volume of supplemented DEF-CS™ medium is prepared by adding DEF-CS™ GF-1 (dilute 1:333), GF-2 (dilute 1:1000) and GF-3 (dilute 1:1000) to DEF-CS™ Basal medium and should be warmed to +37±1°C before use. Discard any left-over warm medium. Warm all other reagents to RT before use.

As a general rule, cells should be seeded at a density of 40-50k/cm^2 (use 40k/cm^2 if leaving the cells 4 days and 50k/cm^2 if leaving 3 days in between passages). This can be adjusted to suit the cell line as appropriate.

When passaging the cells it is highly recommended that the cells are grown to a confluence of 150-300k/cm^2, see pages 6-8 for corresponding images of DEF-hiPSC™ in culture. If cultures should appear suboptimal, it is recommended to increase the seeding density. The passage interval may have to be adjusted accordingly.

Passage
1. Remove medium from cell culture flasks and wash the cell layer once with D-PBS (-/-).
2. Add 20 µL/cm^2 of TrypLE™ Select to the cell culture flasks and incubate for 5 minutes or until the cell layer has detached. Detachment can be aided by swirling the cell culture flask or by tapping the side of the cell culture flask firmly but gently.
3. Resuspend the cells in the supplemented DEF-CS™ medium and pipette up and down several times to ensure a single cell suspension is achieved. (The cells will aggregate if left too long in TrypLE™ Select).

4. After dissociation there is no need to centrifuge the cell suspension to remove TrypLE™ Select if it has been diluted at least 1:10. (Optional: centrifuge the cells at 200g for 2-5 minutes).
5. Count the cells in a haemocytometer or in a cell counter (optimized for each cell type).
6. Add the appropriate volume of cell suspension and medium to the newly coated cell culture flasks to obtain the selected density. The seeding volume of supplemented DEF-CS™ medium should be 0.15-0.2 mL/cm^2.
7. Tilt the flask backwards and forwards gently to ensure the cell suspension is dispersed evenly over the surface and place in the incubator.

Medium Change

Add only DEF-CS™ GF-1 (dilute 1:333) and GF-2 (dilute 1:1000) to DEF-CS™ Basal medium before use. The medium should be warmed to +37±1°C before use. Discard any leftover warm medium.

Medium change is recommended daily (except day of passage), use 0.25-0.4 mL/cm^2.
If the medium colour turns yellow, due to high metabolic activity, the medium volume should be increased.

1. Check cells under microscope; photo document as necessary.
2. Carefully aspirate the medium and pipette newly warmed medium into the cell culture flask. Avoid flushing medium directly on the cell layer.
3. Place the cell culture flask in the incubator.

Freezing of hiPSC Cultured in DEF-CS™

Human induced pluripotent stem cells cultured in DEF-CS™ can be cryopreserved by using common slow freezing protocols for cell suspensions including DMSO and FBS. As a general guide, 2.5-3.5x10^6 cells in 1 mL freezing medium should be frozen in a 2 mL cryo vials.

Transfer from Other Culture Systems

hiPS cells maintained in other culture systems can readily be transferred to DEF-CS™. Fresh cultures can be transferred and also cryopreserved cultures can be thawed directly into the DEF-CS™.
The normal DEF-CS™ culture protocol should be followed although some considerations can be taken into account.

Coating of Culture Vessels with DEF-CS™ COAT-1
When seeding hiPS cells previously cultured in a different culture system, the cells could benefit from a more concentrated coating of DEF-CS™ COAT-1. The normal DEF-CS™ culture protocol stipulates a 1:20 dilution, however for extra support during the transfer process, the dilution ratio can be lowered to e.g. 1:10 or 1:5 for the first passages.

Seeding density
When seeding hiPS cells previously cultured in a different culture system, the cells could benefit from a higher seeding density for the first passages, e.g. 80k/cm^2.

Passage interval
When seeding hiPS cells previously cultured in a different culture system, the cells might initially be growing slightly slower. Depending on the confluence of the cell monolayer, the suitable interval might be between 3 to 7 days for the first passages. The cells should adapt morphology as displayed in the images for 150k/cm^2 and 200k/cm^2 prior to passage. If the cells are still sparse after 7 days in culture a passage is still recommended.

Images of DEF-hiPSC™ in Culture

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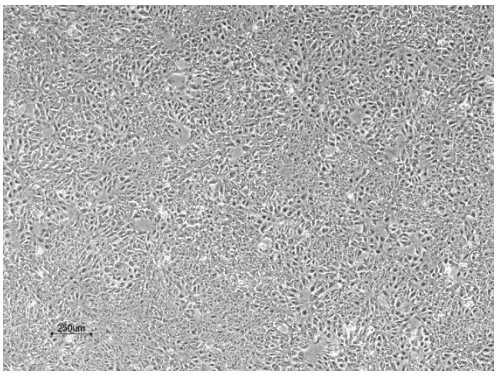
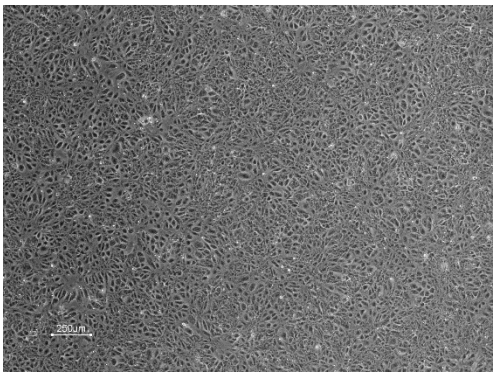
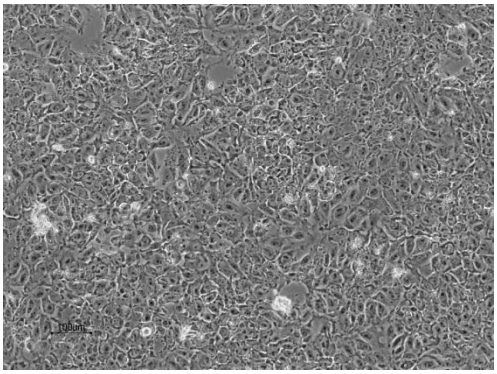
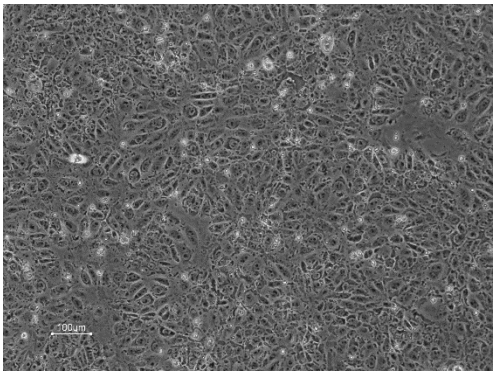
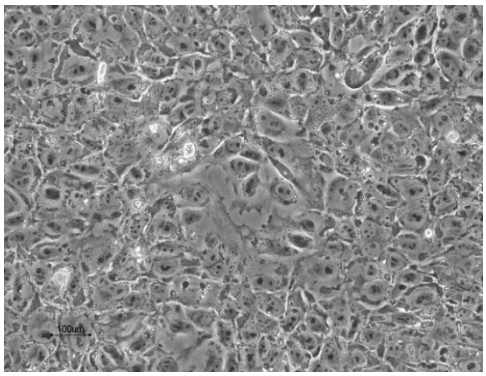
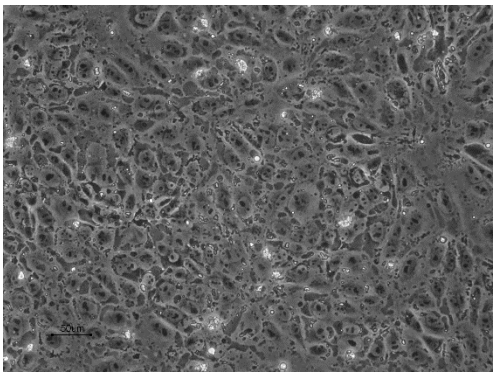
4x		
10x		
20x		

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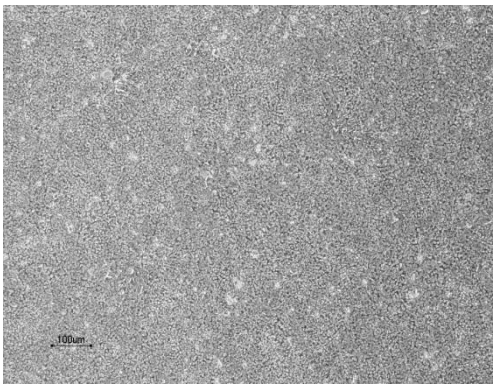
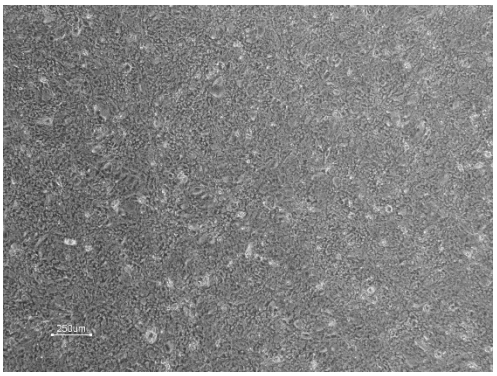
Cell line: DEF-ChiPSC4
Cell density: 150k/cm^2
Flattened homogenous layer

Cell line: DEF-ChiPSC18
Cell density: 150k/cm^2
Flattened homogenous layer

4x





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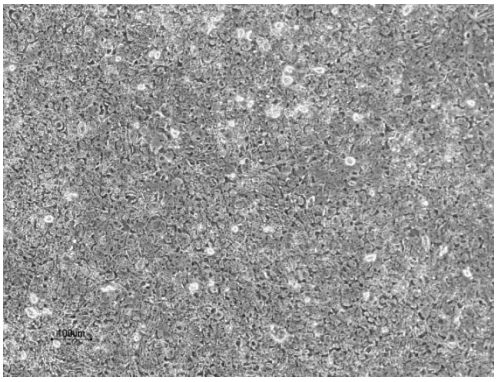

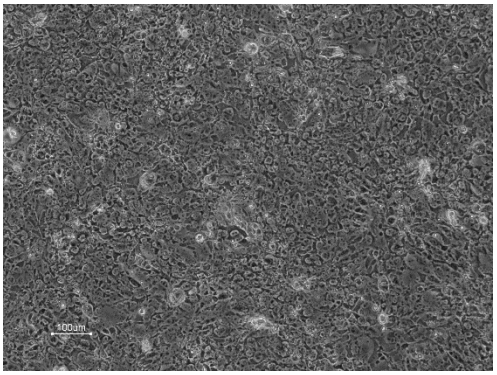

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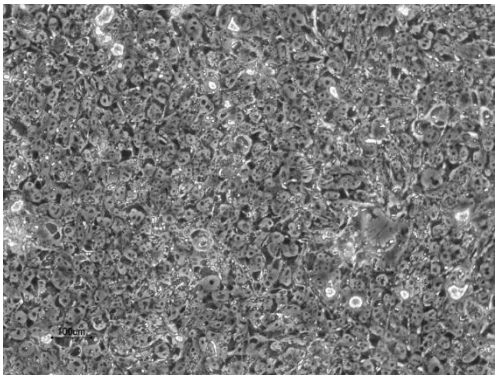

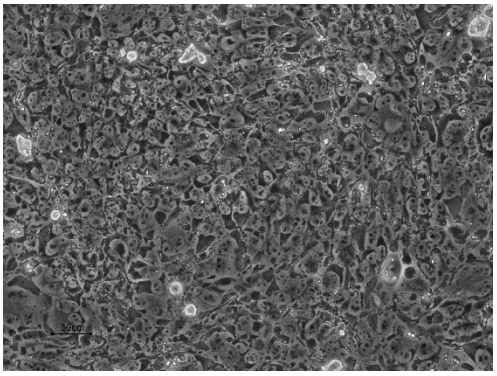

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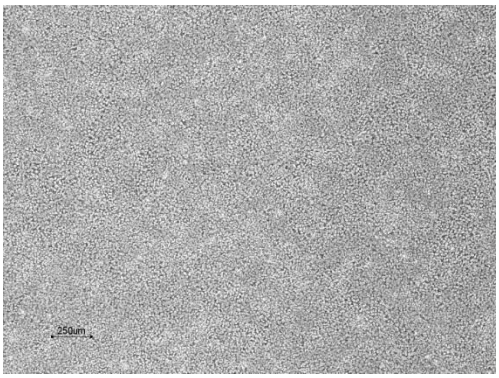
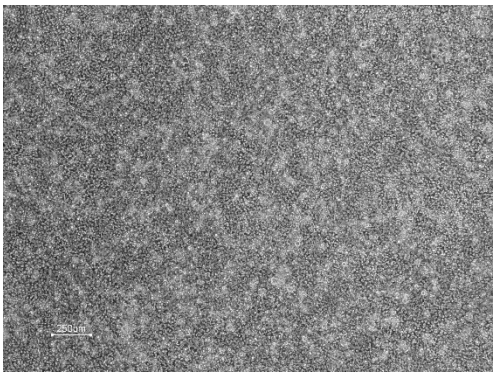
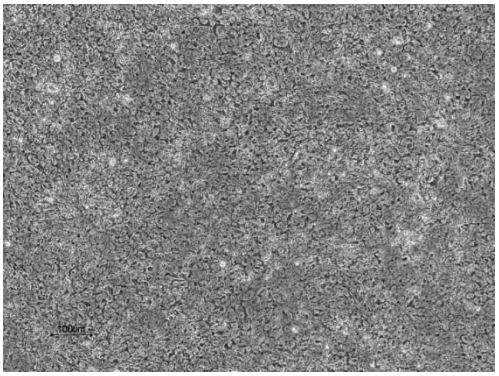
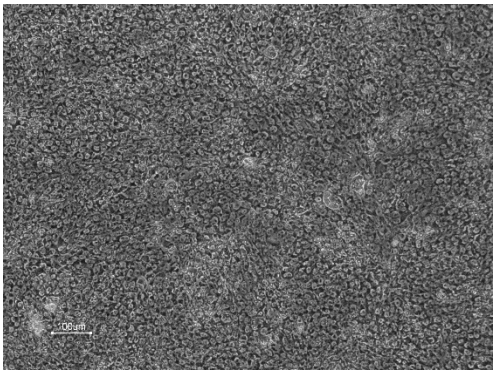
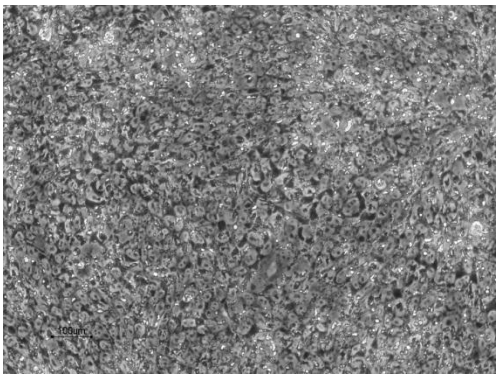
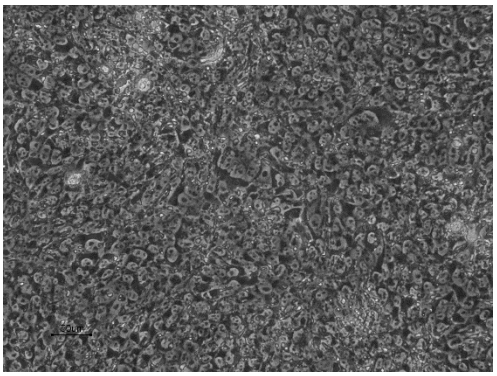
4x		
10x		
20x		

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