

## TECHNICAL MANUAL

# DEF-CS™ - Defined Culture System for maintenance of human pluripotent stem cells (hES and hiPS cells)

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## General information

CATALOGUE #: DEF-501-KIT/Y30010 1 bottle à 500 mL of DEF-CS™ Basal medium (DEF-501-500/Y30011)  
 1 vial à 4 mL of DEF-CS™ COAT-1 Culture Matrix (ACP-604-0004/Y30012)  
 2 frozen vials à 750 µL of DEF-CS™ GF-1 Additive, (ACP-601-0750/Y30013)  
 1 frozen vial à 500 µL of DEF-CS™ GF-2 Additive, (ACP-602-0500/Y30014)  
 1 frozen vial à 200 µL of DEF-CS™ GF-3 Additive, (ACP-603-0200/Y30015)

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

The DEF-CS™ is shipped in a cooled box (DEF-CS™ Basal medium and COAT-1 Culture Matrix) and on dry ice (DEF-CS™ GF-1, GF-2, and GF-3 Additives) 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 Cellartis® DEF-hES™ cell lines (SA121, SA167, SA181 and SA461) and DEF-hiPS™ cells (ChiPSC-4). If you wish to use DEF-CS™ for other 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 <sup>2+</sup> & Mg <sup>2+</sup> (D-PBS +/-)	Gibco/Life Technologies	14040
PBS Dulbecco's w/o Ca <sup>2+</sup> & Mg <sup>2+</sup> (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 Certificate of Analysis.

Preparation of culture medium by addition of DEF-CS™ GF-1, GF-2 and GF-3 additives 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.

The cell expansion capability for 500 mL of DEF-CS™ Basal medium is:  
20x T25 or 8x T75 or 4x T150 flasks.

#### *DEF-CS™ GF-1, GF-2, and GF-3 Additives*

DEF-CS™ GF-1, GF-2 and GF-3 additives 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 Culture Matrix*

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

### Storage and Handling of Frozen DEF-hES™ cells and DEF-hiPS™ cells

The DEF-hES™ cells and DEF-hiPS™ cells are provided frozen in vials and are delivered on dry ice. The vials should be transferred to liquid N<sub>2</sub> when received or thawed according to procedure for thawing of frozen DEF-hES™ cells.

DEF-hES™ cells and DEF-hiPS™ cells 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<sup>2</sup>. The DEF-hES™ cells and DEF-hiPS™ cells should be maintained in an incubator at +37±1°C, 5 % CO<sub>2</sub> and > 90% humidity.

### Coating of Cell Culture Flasks

1. Dilute the required volume of DEF-CS™ COAT-1 Culture matrix in D-PBS (+/+) before use. Make a 1:20 dilution.
2. Mix the diluted DEF-CS™ COAT-1 Culture matrix solution gently and thoroughly by pipetting up and down.
3. Add the appropriate volume of diluted DEF-CS™ COAT-1 Culture matrix solution to the cell culture flasks (use 0.1 mL/cm<sup>2</sup>), 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.
5. Aspirate DEF-CS™ COAT-1 Culture matrix solution from cell culture flasks just before use.
6. The cell culture flasks are now coated and can be used for DEF-hES™ or DEF-hiPS™ cell culture.

## Thawing of frozen vials DEF-hES™ cells and DEF-hiPS™ cells

### Preparation

Cell culture units should be coated as described above before use.

The appropriate volume of DEF-CS™ Basal medium is supplemented with DEF-CS™ GF-1 (dilute 1:333), GF-2 (dilute 1:1000) and GF-3 (dilute 1:1000) additives, at day of use and should be warmed to +37±1°C before use. Discard any left-over warmed 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 10 mL of the DEF-CS™ Basal medium with supplements to a centrifuge tube.
2. Using forceps, transfer vial directly into a container with +37±1°C water. Thaw the vial by gently pushing it under the surface of the water. Never hold the vial in your hand because doing so poses an explosion risk.
3. Once thawed, decontaminate the vial in appropriate disinfectant.
4. Pipette the contents of the vial into the sterile tube containing 10 mL supplemented DEF-CS™ Basal medium.
5. Centrifuge at 300g for 1 minute.
6. After centrifugation, aspirate the supernatant and re-suspend the pellet in 3 mL supplemented DEF-CS™ Basal medium.
7. Pipette the cell suspension into appropriate cell culture unit of 10 cm<sup>2</sup> in total cell culture area.
8. 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-hES™ and DEF-hiPS™ cells

Cell culture flasks should be coated as described above before use.

DEF-CS™ Basal medium is supplemented with DEF-CS™ GF-1 (dilute 1:333), GF-2 (dilute 1:1000) and GF-3 (dilute 1:1000) additives and should be warmed to +37±1°C before use. Discard any left-over warmed medium. Warm all other reagents to room temperature before use.

As a general rule, cells should be seeded at a density of 40-50k/cm<sup>2</sup> (use 40k/cm<sup>2</sup> if leaving the cells 4 days and 50k/cm<sup>2</sup> 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<sup>2</sup>, see pages 6-8 for corresponding images of DEF-hES™ and DEF-hiPS™ cells in culture. If cultures should appear suboptimal, it is recommended to increase the seeding density. The passage interval may have to be adjusted accordingly.

1. Remove medium from cell culture flasks and wash the cell layer once with D-PBS(-/-).
2. Add 20 µL/cm<sup>2</sup> 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. Re-suspend the cells in the supplemented DEF-CS™ Basal medium and pipette up and down several times to ensure a single cell suspension is achieved. (The cells will re-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™ Basal medium should be 0.15-0.2 mL/cm<sup>2</sup>.
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) additives to DEF-CS™ Basal medium before use. The medium should be warmed to +37±1°C before use. Discard any leftover warmed medium.

Medium change is recommended daily (except day of passage), use 0.25-0.4 mL/cm<sup>2</sup>. 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 DEF-hES™ and DEF-hiPS™ cell suspension

The DEF-hES™ cells and DEF-hiPS™ cells can be cryopreserved by using common slow freezing protocols for cell suspensions including DMSO and FBS. As a general guide, 2.5-3.5x10<sup>6</sup> cells in 1 mL freezing medium should be frozen in a 2 mL cryo vials.

### Transfer from other culture systems

hES and 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 Culture matrix*

When seeding hES cells or hiPS cells previously cultured in a different culture system, the cells could benefit from a more concentrated coating of DEF-CS™ COAT-1 Culture matrix. 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 hES cells or 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<sup>2</sup>.

#### *Passage interval*

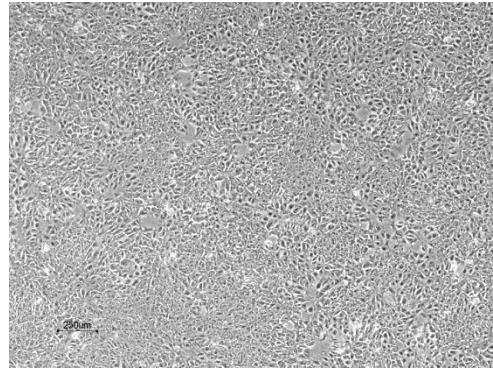
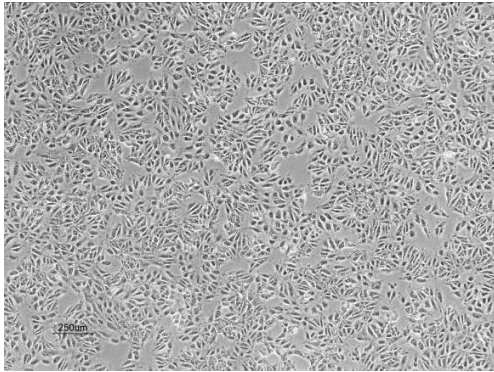
When seeding hES cells or hiPS cells previously cultured in a different culture system, the cells might initially be growing slightly slower. Depending on the confluence of the cell monolayer, the suitable interval might be between 3 to 7 days for the first passages. The cells should adapt morphology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

**Images of DEF-hES™ (left column) and DEF-hiPS™ (right column) cells in culture**

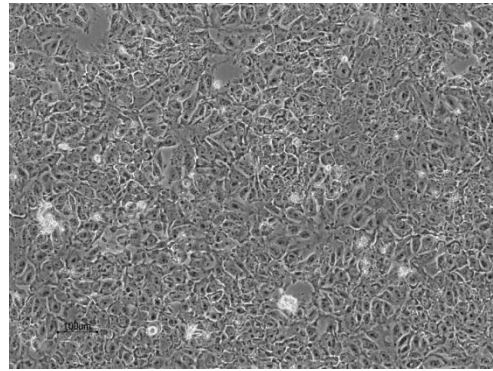
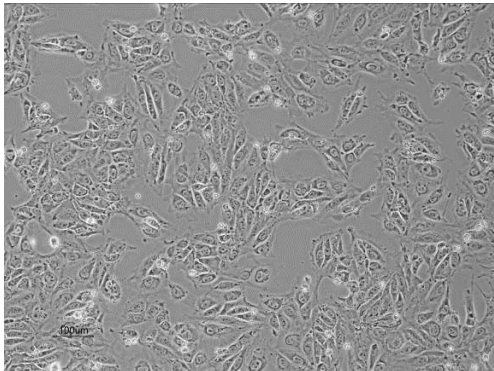
Cell line: DEF-SA121  
Cell density: 50k/cm<sup>2</sup>  
Flattened homogenous layer

Cell line: DEF-ChiPSC4  
Cell density: 50k/cm<sup>2</sup>  
Flattened homogenous layer

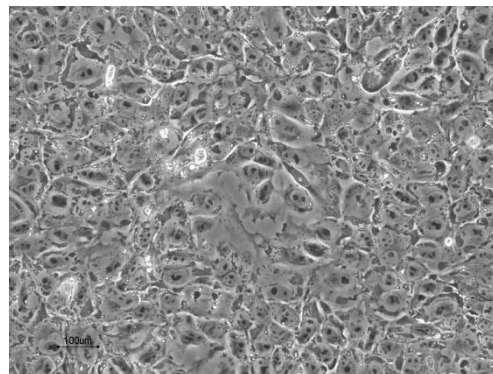
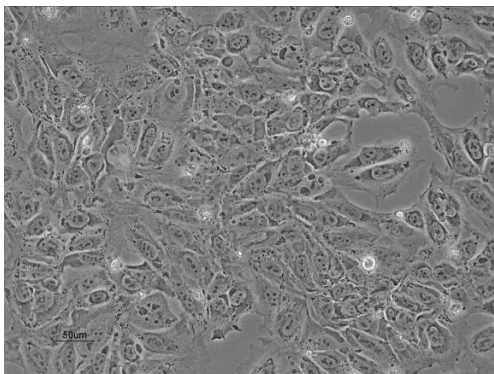
4x



10x



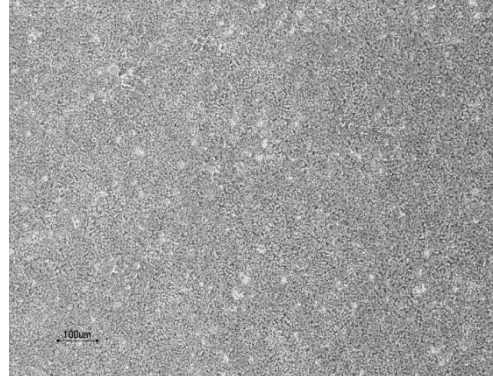
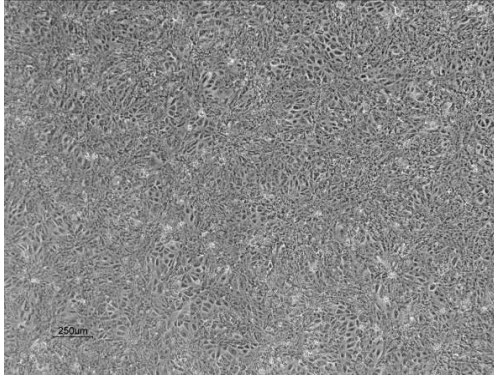
20x



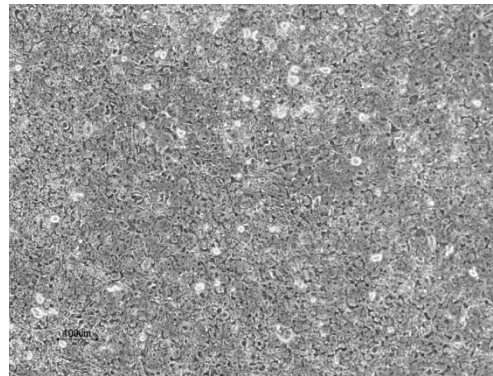
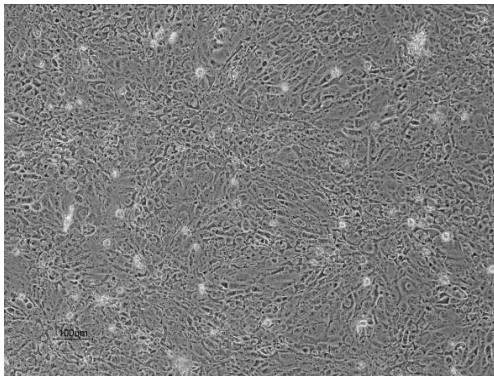
Cell line: DEF-SA121  
Cell density: 150k/cm<sup>2</sup>  
Flattened homogenous layer

Cell line: DEF-ChiPSC4  
Cell density: 150k/cm<sup>2</sup>  
Flattened homogenous layer

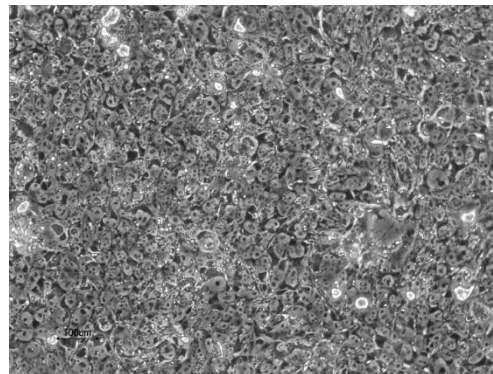
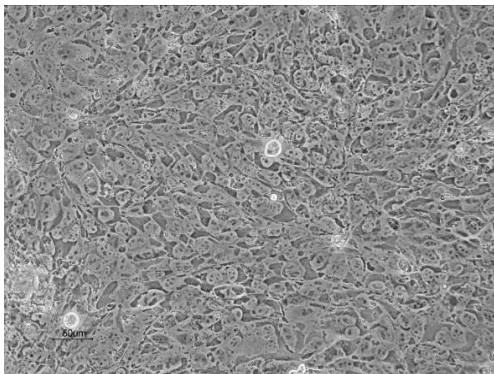
4x



10x



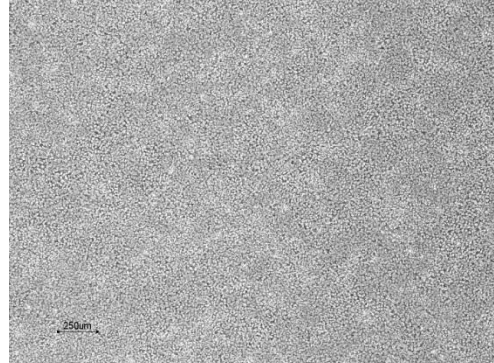
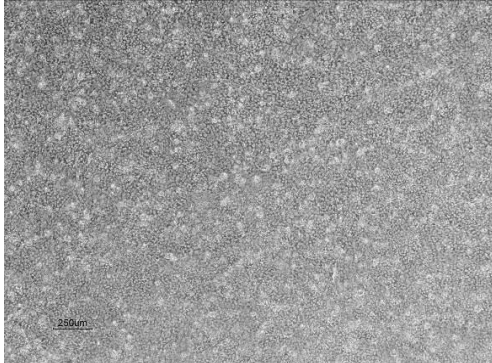
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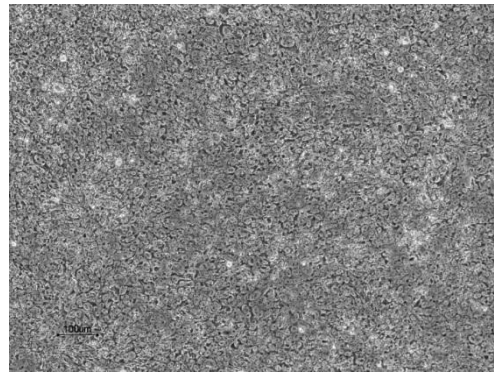
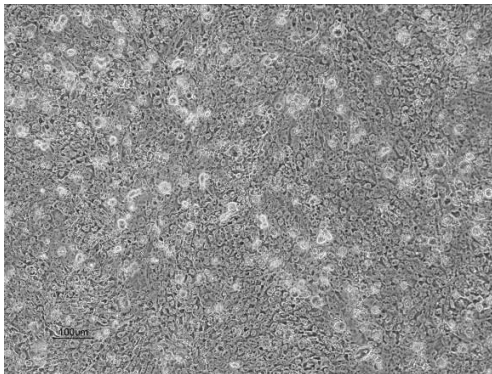
Cell line: DEF-SA121  
 Cell density: >200k/cm<sup>2</sup>  
 Flattened homogenous layer

Cell line: DEF-ChiPSC4  
 Cell density: >200k/cm<sup>2</sup>  
 Flattened homogenous layer

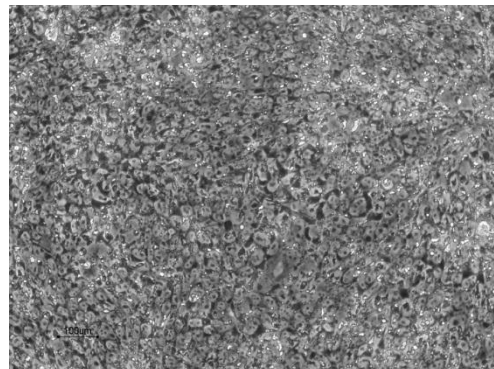
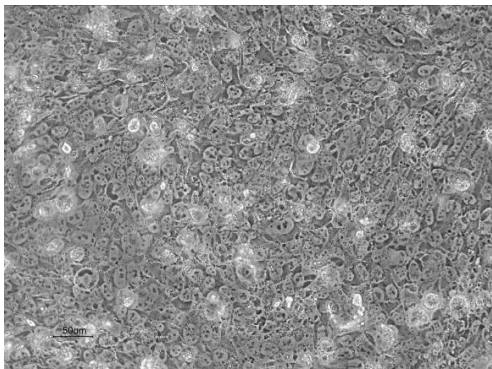
4x



10x



20x



For technical support email: [tech-cellartis@takara-clontech.eu](mailto:tech-cellartis@takara-clontech.eu)

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#### Authorised uses

Except as otherwise agreed in writing, the purchase of goods only conveys to you the non-transferable right for only you to use the quantity of goods and components of goods purchased in compliance with the applicable intended use statement. Unless otherwise authorized, no right to resell the goods, or any portion of them, is conveyed hereunder.

The goods are intended for research use only and are not to be used for any other purposes including, but not limited to: unauthorized commercial purposes, *in vitro* diagnostic purposes, *ex vivo* or *in vivo* therapeutic purposes, investigational use, in foods, drugs, devices or cosmetics of any kind, or for consumption by or use in connection with or administration or application to humans or animals.