User Guide Plus (Acclimation in iPSC culture)

Ceglu™ cultureware

Introduction

When transitioning cells from other matrix environments to Ceglu™ multiwell plates, acclimation is recommended. This process helps stabilize subsequent maintenance cultures and supports consistent differentiation. User Guide Plus includes an example of a suitable seeding density study for iPSC culture.

Acclimation process

Step 1: 1st passage of seeding density conditions

In the 1st passage, it is recommended testing a broad range of seeding densities, including high-density conditions. This helps identify optimal conditions for cell attachment and growth.

Example conditions：3 x 10^4 cells/well, 6 x 10^4 cells/well, 9 x 10^4 cells/well

Notes: Cells may temporarily undergo increased cell death due to adaptation stress caused by changes in the culture environment.

Step 2: 2nd passage of seeding density conditions

For the 2nd passage, collect cells from wells that showed favorable growth in the 1st passage. We recommend further refining the seeding density conditions based on these results. Please refer to the acclimation example on the reverse side for more details.

During the early stages of acclimation, spontaneously differentiated cells may appear. However, as acclimation progresses, the number of differentiated cells typically decreases. Acclimation usually takes about 2–3 weeks. Completion of acclimation is determined by the stabilization of cell morphology and proliferation rate.

Reference information:

Cells acclimated on Ceglu™ are recommended to undergo differentiation using the same Ceglu™ platform. Since differentiation can be performed within the same matrix environment, users can simplify their protocols. Ceglu™-acclimated cells have demonstrated the ability to differentiate into all three germ layers and confirmed their potential to generate a wide variety of cell types.

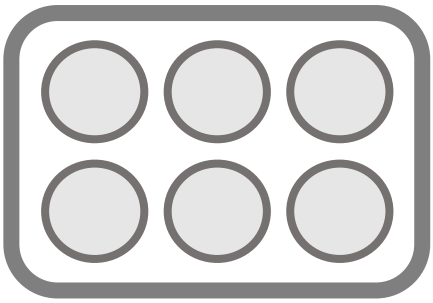
Examples: Neural progenitor cells, Astrocytes, Cardiomyocytes, Hematopoietic progenitor cells, Hepatoblast, and others

For more information, please refer to the Application Notes available on the Ceglu™ product website.

https://www.sekisui-cell.jp/en/products/ceglu/

Example of Acclimation

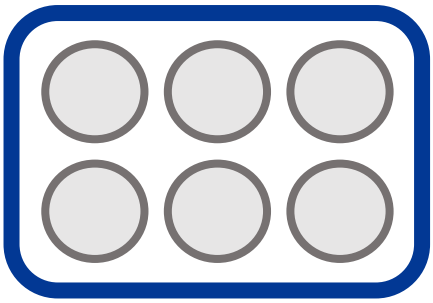
Other matrix



Frozen cell stocks can also be used

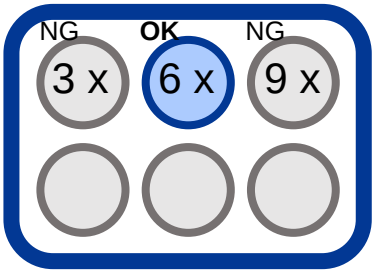
Acclimation on Ceglu™

Ceglu™



Optimizing the seeding density for Ceglu™ is essential. Modifications to the initial seeding density may be necessary to achieve optimal cell performance.

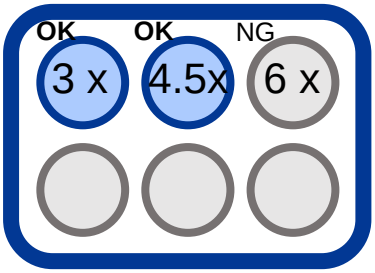
1st



Seeding densities between 3 × 10^4 and 1 × 10^5 cells per well are suitable for use with Ceglu™. It is recommended to evaluate several conditions within this range to determine the most effective density for your specific application.

Passage

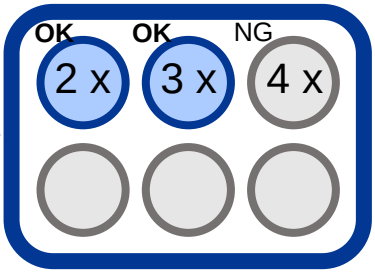
2nd



Since culturing at 6 × 10^4 cells per well was successful during the initial passage, it is appropriate to maintain this condition while also considering lower seeding densities in anticipation of accelerated cell proliferation. As the cells begin to acclimate to Ceglu™, successful culture has also been achieved at a lower density of 3 × 10^4 cells per well.

Passage

3rd



During the 2nd passage, the previously successful condition of 6 × 10^4 cells per well was maintained. As acclimation to Ceglu™ progressed and cell proliferation was expected to accelerate further, a new condition of 2 × 10^4 cells per well was introduced. The 3 × 10^4 cells per well condition was also retained. Under these circumstances, seeding densities in the range of 2–3 × 10^4 cells per well appear to be suitable.

Acclimation to Ceglu™ is generally achieved within 2 to 3 passages. Stabilization of cell morphology and proliferation rate are key indicators of successful acclimation.

Note: Depending on the cell line, a longer acclimation period may be required.

For more information about Ceglu™ and our other products, please visit our website.

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