

## Pluripotent Stem Cells (iPSC and ESC) *Expansion Protocol for CERO*



### • Introduction

CERO provides a unique approach for lab-scale production of induced pluripotent stem cells (iPSC) and embryonic stem cells (ESC).

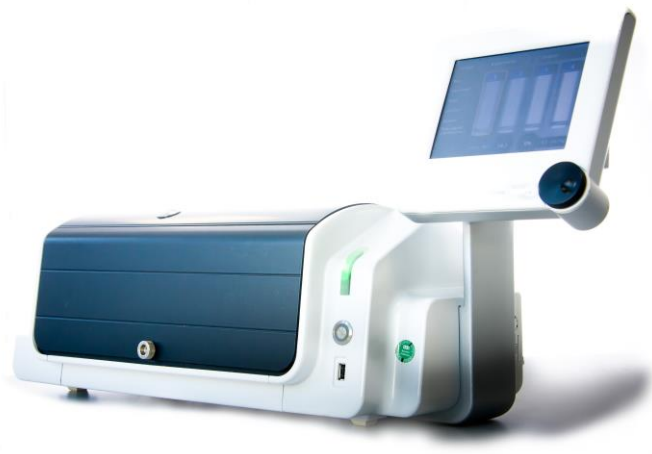
- + Microcarrier free
- + Free floating, monodisperse 3D aggregates
- + No spontaneous differentiation
- + Stable pluripotency
- + Flexible inoculation density
- + Maximal cell yield

CERO provides a simple, reproducible and very efficient way to generate **high yield of pluripotent stem cells**.

CERO 3D culture of pluripotent stem cells enables cultivation of homogenous cell populations to be used in various applications like **biobanking**, **cell-based drug development**, **toxicity testing**, **regenerative medicine** and more.

CERO is a unique platform for **testing disease- and patient specific cell types**, serving as a model for studying the cellular and molecular phenotype of diseases.

CERO enables generation of homogeneous iPSC and ESC aggregates that can easily be used in 2D downstream applications.



• **Standard Protocol<sup>(1)</sup>**

**I. 2D Pre-cultivation of Pluripotent Stem Cells (PSC)**

1. Culture PSC in PSC Medium, supplemented with PSC Supplement
2. Split PSC once in 4 days; ratio 1:6

**II. 3D PSC Aggregate Formation – Inoculation**

1. Detach PSC with Accutase solution
2. Count and pellet PSC
3. Inoculate PSC into CERO at recommended concentrations in 10ml PSC Medium supplemented with 10 µM Y-27632 (Rho-Kinase Inhibitor)

Recommended initial cell concentrations for inoculation of PSC in CERO

Species \ cell conc. per ml	Tube 1	Tube 2	Tube 3	Tube 4
Non Human	15.000	30.000	45.000	60.000
Human	30.000	45.000	60.000	80.000

CERO “**Inoculation**” settings:

Rotation			Agitation		Protocol
speed (rpm)	time (sec)	pause (sec)	period (min)	pause (min)	duration
80 <sup>(2)</sup>	5	2	0	0	12 h

12h after inoculation select initial cell concentration based on homogeneity and size of aggregates.

**III. PSC Aggregate Expansion – Cultivation**

Stop rotation and leave the CERO Tube in the CERO for 5 to 10 min. to allow organoids to settle down

1. Add 5ml of PSC Media every 24 hours (media conditioning)

CERO “**Cultivation**” settings:

Rotation			Agitation		Protocol
speed (rpm)	time (sec)	pause (sec)	period (min)	pause (min)	duration
80 <sup>(2)</sup>	1	2	0	0	∞

2. After 3 - 4 days stop rotation and continue with harvesting step

To continue with the generation of organoids in CERO, please continue as described in



“Organoids from pluripotent stem cells - Differentiation Protocol”.

For expansion of PSC, continue with harvesting step.

**IV. PSC Aggregate Dissociation – Harvesting**

1. After stop of rotation at end of cultivation, leave the CERO Tube in the CERO for 5 to 10 min. to allow PSC aggregates to settle down
2. Carefully aspirate the supernatant without disturbing the pellet
3. Wash pellet one time with 15ml PBS without Ca and Mg
4. Carefully aspirate the PBS without disturbing the pellet
5. Add ~ 10ml Accutase (or other dissociation reagent) and incubate using the following settings.

CERO “**Harvesting**” settings:

Rotation			Agitation		Protocol
speed (rpm)	time (sec)	pause (sec)	period (min)	pause (min)	duration
80 <sup>(2)</sup>	1	2	0	0	15

6. Transfer the single cell suspension of PCS into Falcon tube and spin cells down at 1300rpm for 3 min.
7. Carefully aspirate the supernatant without disturbing the pellet
8. Carefully snip the pellet and resuspend in the PSC Media containing 10 µM Y-27632 (Rho-Kinase Inhibitor)

After harvesting, the cells can be frozen for storage or used in assays. For further expansion, continue with **II step 3** of inoculation.

- **Request more information**  
info@ols-bio.de | www.ols-bio.de  
+49-421-2761690

<sup>1</sup>Note : Make sure CERO is connected to CO<sub>2</sub> and use general settings 37°C with 5% CO<sub>2</sub>.

<sup>2</sup>For human cells. For non-human cells, rotation speed up to 100 rpm is feasible.