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).

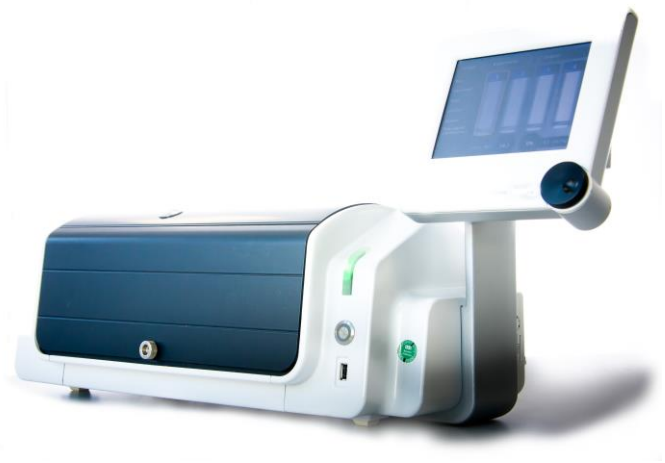
+ Microcarrier free
+ Free floating, monodisperse 3D aggregates
+ No spontaneous differentiation
+ Stable pluripotency
+ Flexible inoculation density
+ Maximal cell yield

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[1]

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[2]	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[2]	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[2]	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**

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[1]Note : Make sure CERO is connected to CO_2 and use general settings 37°C with 5% CO_2.
[2]For human cells. For non-human cells, rotation speed up to 100 rpm is feasible.