OMNI Life Science

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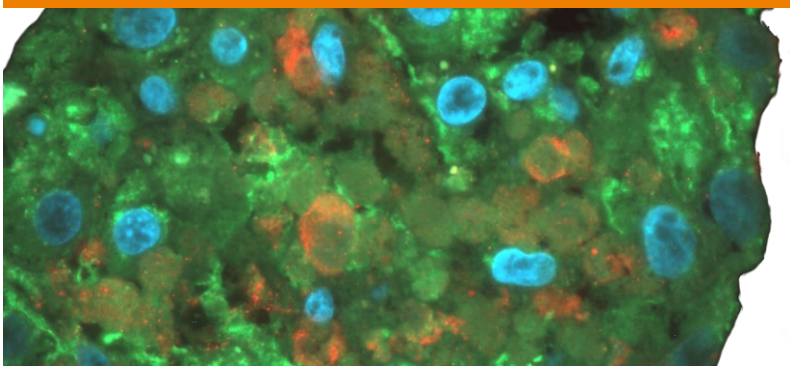
Organoids from Pluripotent Stem Cells

Differentiation Protocol for CERO



Introduction

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

+ Brain, cardiac, hepatic, epithelial organoids and more
+ 3D - free floating organoids
+ No harmful shear forces
+ Low demand in Matrigel
+ Minimized levels of necrosis
+ High yield and less cost
+ Homogeneous, identical conditions for up to 4000 organoids in a single instrument
+ Stable long-time differentiation and survival (1 year and more)

Generating organoids can vary due to type of stem cells used and targeted type of resulting organoids. This protocol describes the optimized process in CERO for the generation of organoids.

Standard Protocol[1]

The protocol starts with monodispersed 3D aggregates of pluripotent stem cells generated in CERO as described in

"Pluripotent Stem Cells (iPSC and ES) - Expansion Protocol"

If organoids have been aggregated in cell culture plates, please skip step I and continue with step II by transferring organoids into CEROtubes.

I. Organoid – Induction

1. *Stop rotation of PSC aggregates (as described in "Pluripotent Stem Cell (iPSC and ES)- Expansion Protocol" and 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 once with 15ml PBS without Ca and Mg*
4. *Carefully aspirate the PBS without disturbing the pellet*
5. *Add 15ml of Induction Media and incubate as described below*

*CERO "**Cultivation**" settings:*

Rotation			Agitation		Protocol
speed (rpm)	time (sec)	pause (sec)	period (min)	pause (min)	duration
80[2]	1	2	0	0	∞

6. *Change Media twice a week or as needed. As a guideline, exchange of media is usually less often required as in 2D Culture. It is recommended to change the CEROtube once a week.*

Perform Induction for 7 to 12 days.

II. Organoid – Differentiation

1. *Stop rotation and leave the CEROtube in the CERO for 5 to 10 min. to allow organoids to settle down*
2. *Carefully aspirate the supernatant without disturbing the organoids*
3. *Wash organoids once with 15ml PBS without Ca and Mg*
4. *Carefully aspirate the PBS without disturbing the organoids*
5. *Add Differentiation Media as described below:*

Number per tube / Size	5-200µm	201 - 500µm	501 - >1000µm
20 - 50	3-7 ml	5-10 ml	15-20 ml
100 - 200	5-10 ml	10-20 ml	20-30 ml
500 - 1000	10-20 ml	20-30 ml	40-50 ml

*CERO "**Cultivation**" settings:*

Rotation			Agitation		Protocol
speed (rpm)	time (sec)	pause (sec)	period (min)	pause (min)	duration
80[2]	1	2	0	0	∞

Change Media once a week or as needed referring to pH following steps below. Please use color changes of the media as an indication. Alternatively add 30ml of media and control pH Value online. As a guideline, exchange of media is usually required half as often as in 2D Cultures.

6. *Stop rotation and leave the CEROtube in the CERO for 5 to 10 min. to allow organoids to settle down*
7. *Carefully aspirate the supernatant without disturbing the organoids and add fresh media according to guideline below:*

replaced media	frequency per week
100%	1
80%	2
50%	3
30%	3-4

Cultivation can be continued long term. As a guideline, a year or longer can normally be achieved easily. Depending on size of organoids, increasing the viscosity on the media may support long term cultures. The minimum period should be in the range of 7 to 12 days. It is recommended to change the CEROtube once a week.

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.