PrimeSurface®

User guide

3D cell culture

In vitro 3D cell culture can mimic *in vivo* microenvironment compared to 2D culture and cells respond more accurately in 3D format. Cells formed from 3D cell culture show higher cell activity compared to normal 2D culture and have been used in many research areas like cancer biology, hepatotoxicity and stem cell biology.

3D spheroid generating PrimeSurface plates are coated with hydrophilic polymer and available in 2 different well bottom designs and the well surface is coated with hydrophilic polymer. These features allow highly reproducible growth of 3D cell cultures for different type of cells

Basic cell culture method

Start with a preliminary experiment to determine the initial seeding density because spheroid formation depends on several factors such as cell type, culture period, and the desired spheroid size.

Depending on the plate type and well dimension, starting media volume is suggested below:

96-well (Cat: MS-9096UZ): 100 to 150 µL per well (recommendation: 125 µL)
384-well (Cat: MS-9384UZ): 35 to 65 µL per well (recommendation: 50 µL)

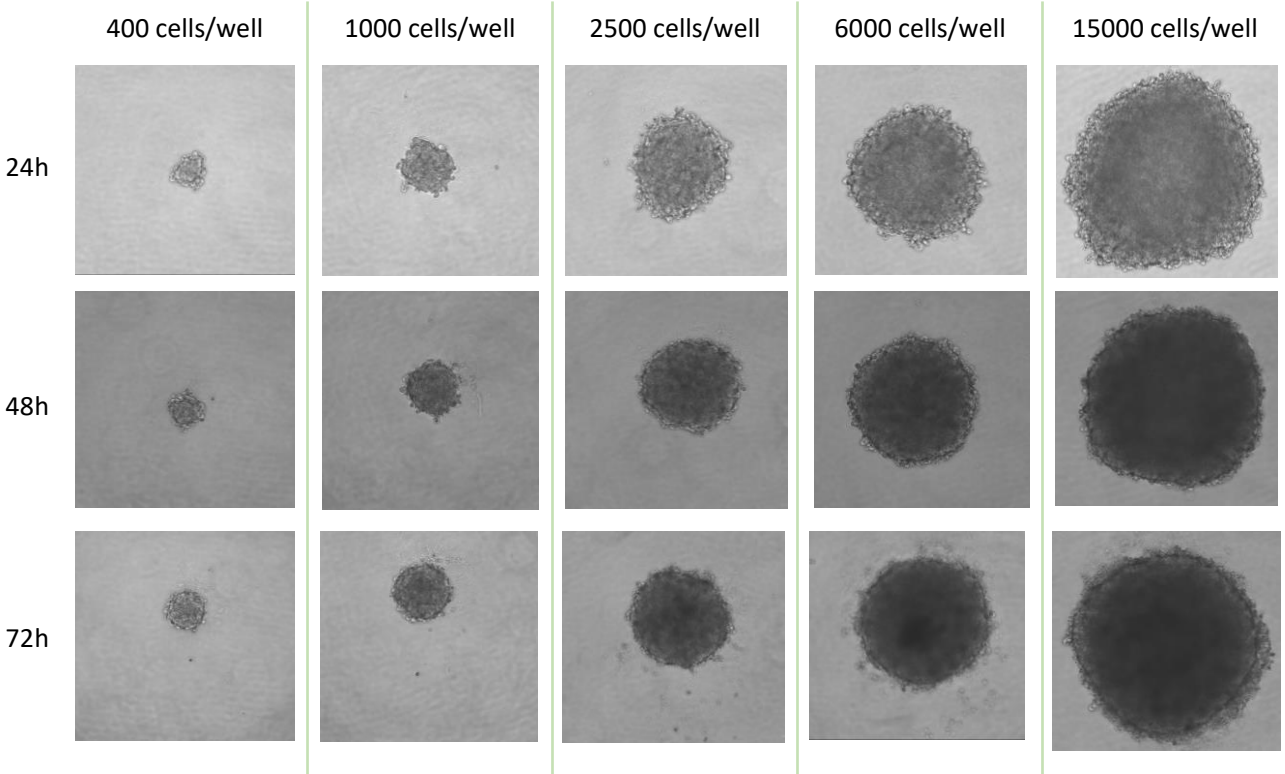
1. Prepare single cell suspension from either frozen vials or fresh cultures at the desired seeding concentration.
2. Dispense cell suspension into each well using a pipettor, multichannel pipettor or dispenser.
   ※ As the surface of each well is coated with hydrophilic polymer, it is important to note that pipet tips do not scratch the bottom or sides of the wells during seeding.
3. After dispensing, cover the plates and transfer to incubation. For most mammalian cultures, set humidified incubator to 37℃ and 5% CO_2.
4. Spheroid formation and growth can be can be monitored daily using any microscope.
   ※Duration of growth phase in a spheroidal format depends on cell type, some cell lines form spheroids within 24 hours and some cell lines need 48 hours or more.
5. Depending on the cell types, media changes may be necessary during spheroid formation.
   ※We recommend that half the media from the plate be removed and replace with fresh media.
   Critical to note that spheroids not be touched, disturbed or mistakenly pipetted out.
   ※If performing a long term cell culture such as organoid formation using iPS cells, 96 slit well plate (Cat: MS-9096SZ) is highly recommended.
6. Perform downstream assay if the spheroids reach the desired size or format.

Examples of spheroids in PrimeSurface cell culture plates

Uniform, single multicellular spheroid can be easily formed from one well

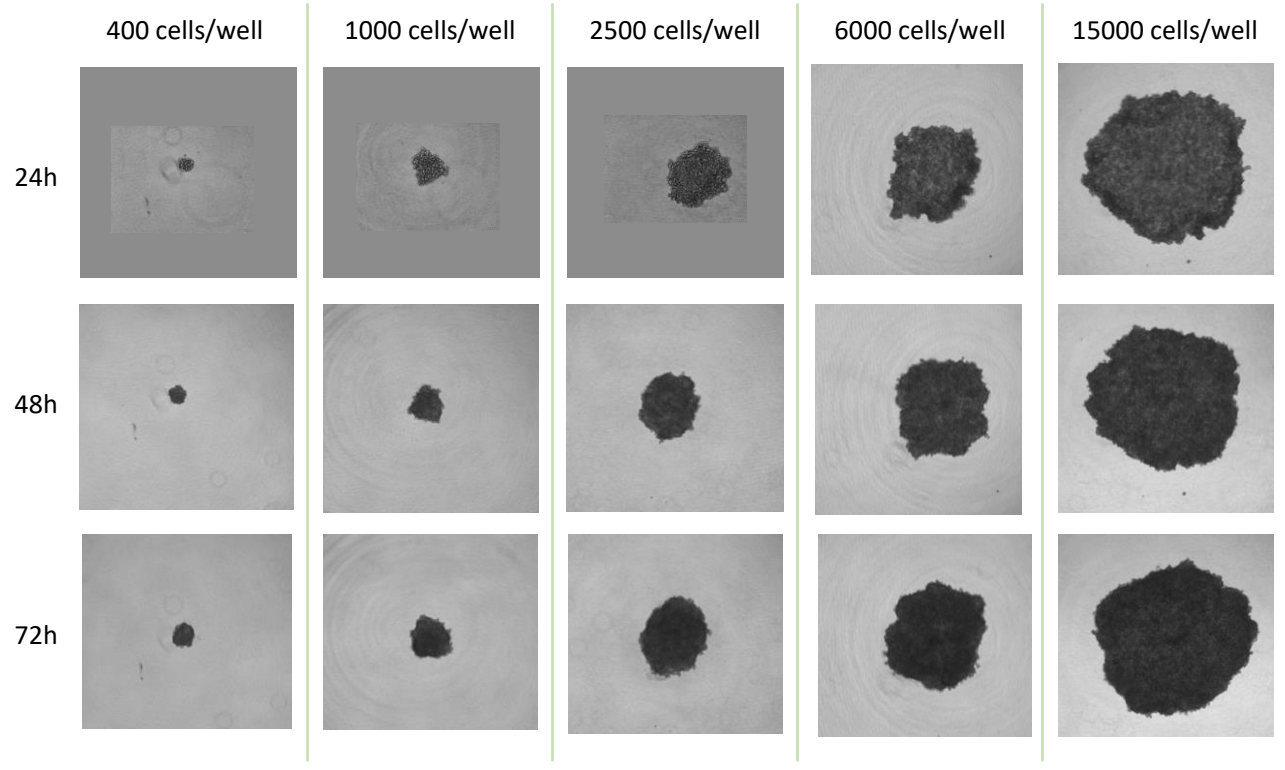
- HeLa spheroids in a 96-well PrimeSurface cell culture plate (Cat: MS-9096UZ)

Scale bar: 200μm



- HepG2 spheroids in a 96-well PrimeSurface cell culture plate (Cat: MS-9096UZ)

Scale bar: 200μm



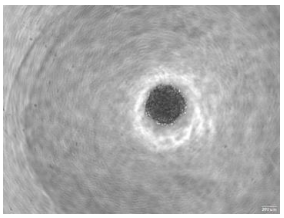
Comparison data

PrimeSurface has the advantage of supporting single well single spheroid formation in uniform size

Cell line: HepG2
Seeding density: 1000 cells/well
Medium: DMEM+10%FBS　Culture period: 3 days
Plate: 96 well ULA plates for 3D culturing from different companies

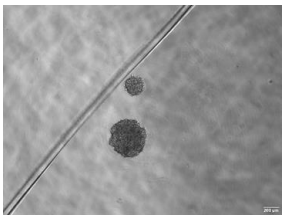
Classification based on microscopic images

<Grade1>



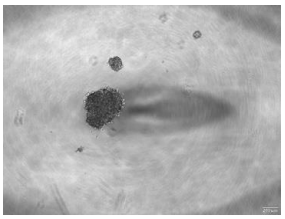
Single spheroid from single well

<Grade2>



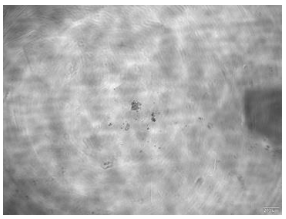
One main spheroid and one small satellite from one well

<Grade3>



3 or more spheroids or badly deformed ones from one well

<Grade4>



No spheroid formation

Comparison of performance of PrimeSurface plates with other ULA plates

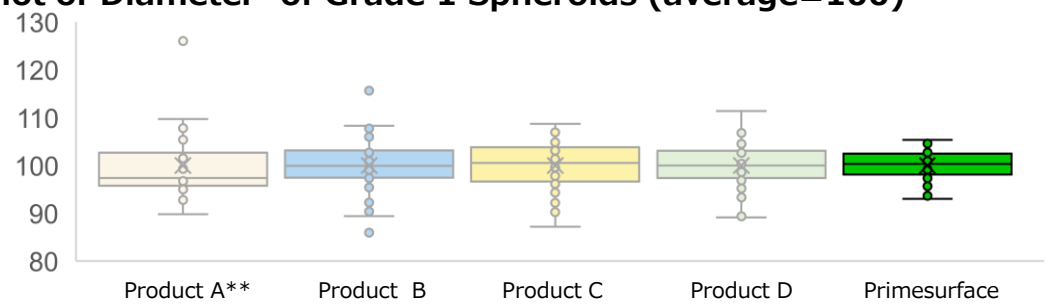
Manufacturer	Product	Grade1	Grade2	Grade3	Grade4
Company A	Product A	23	3	70	0
Company B	Product B	65	27	4	0
Company C	Product C	83	8	5	0
Company D	Product D	88	6	1	1
S-BIO Sumitomo Bakelite Co., Ltd.	**PrimeSurface**	**96**	**0**	**0**	**0**

*Diameter was calculated from the Day3 spheroid area, assuming that spheroid was sphere
**Data of Product A was from Day2 spheroids

Diameter* of Grade 1 Spheroids

	Product A** (n=23)	Product B (n=65)	Product C (n=83)	Product D (n=88)	**PrimeSurface** (n=96)
Ave (μm)	450.7	488.9	509.5	477.4	**480.7**
SD	32.9	22.6	23.4	20.2	**14.6**

Box plot of Diameter* of Grade 1 Spheroids (average=100)

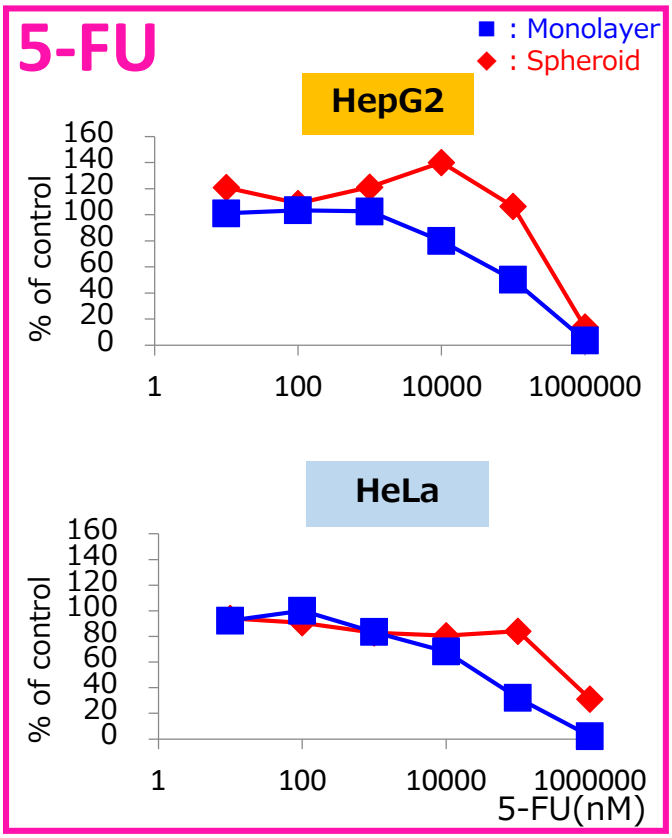


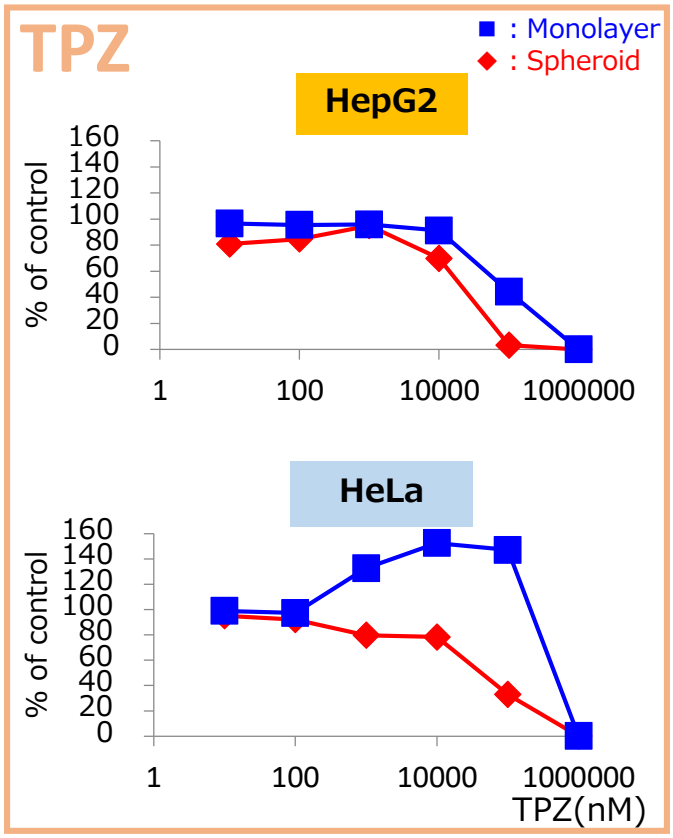
Spheroid assay ~Anticancer drug screening~

Comparison of drug efficacy between Monolayer and Spheroid using anticancer drugs with different MOA

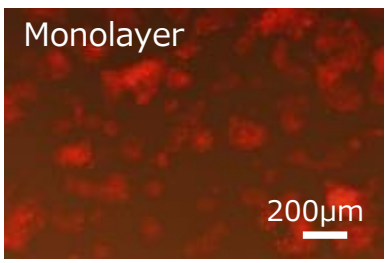
5-Fluorouracil (5-FU):
universal DNA damaging cytotoxic drug
Tirapazamine (TPZ):
hypoxia triggered DNA damaging cytotoxic drug

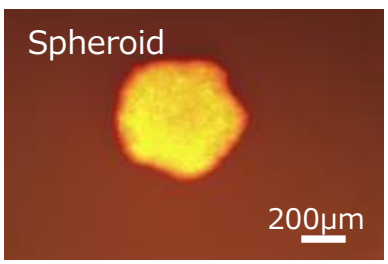
Seeding density: 1500 cells/well
Medium: DMEM+10%FBS
Drug exposure: 48 h from Day4

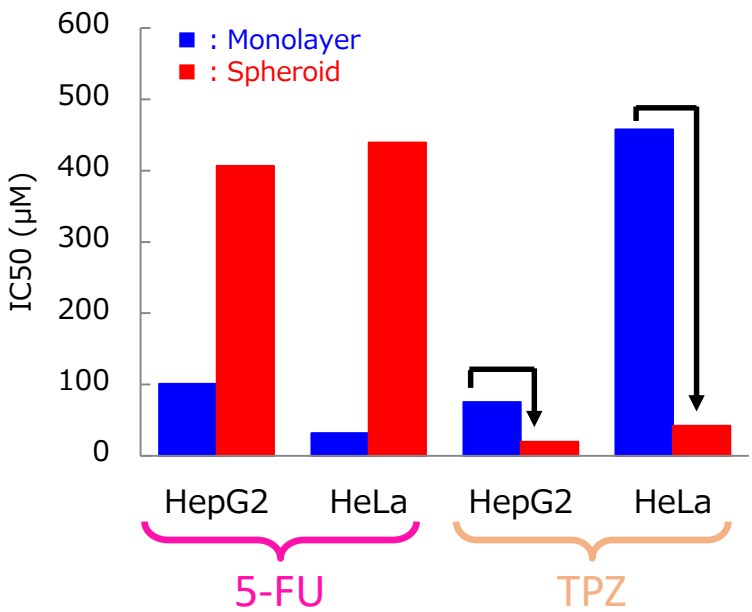




Hypoxia Observation in HepG2 Spheroid with Lox-1 Probe







TPZ showed stronger drug efficacy in spheroids than monolayer cultured cells.

Different well bottom designs of PrimeSurface

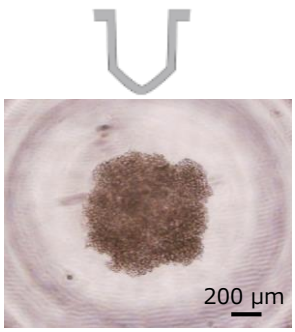
+ V bottom plate successfully supports aggregation of the cancer cells which exhibit low cohesive force .

Cell line: MDA-MB-468
Seeding density: 2000 cells/well

Medium: RPMI+10%FBS
Culture period: 7 days

MS-9096UZ

MS-9096VZ



Data provided by:
Professor Kazuto Nishio
Dept. of Genome Bio.
Faculty of Medicine
Kindai University

Brain organoids generation from iPS cells

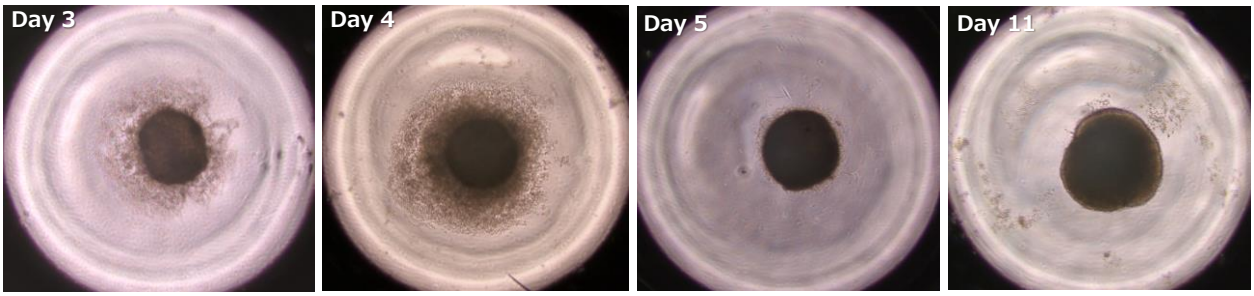
+ V bottom plate is suitable for human iPS cell aggregation for efficient self-organization and organoid formation.

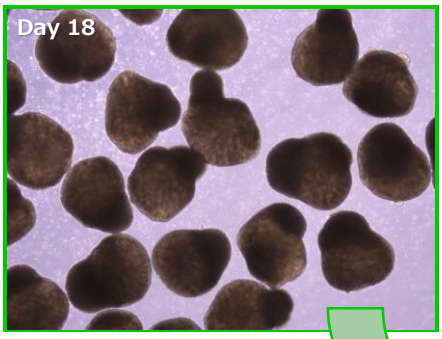
Seeding density : 10000 cells/well

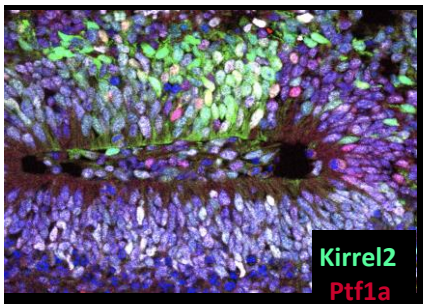
Scale bar: 500 µm

Data provided by:
Professor Keiko Muguruma
Faculty of Medicine
Kansai Medical University

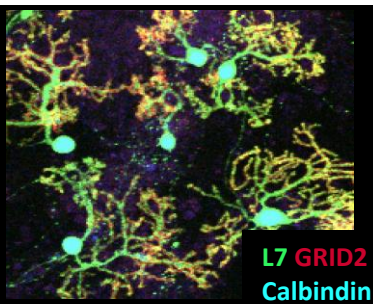
【 Cerebellar 】





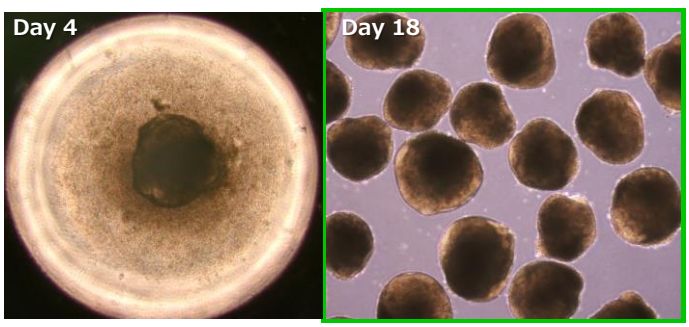


Cerebellar organoid on Day 35

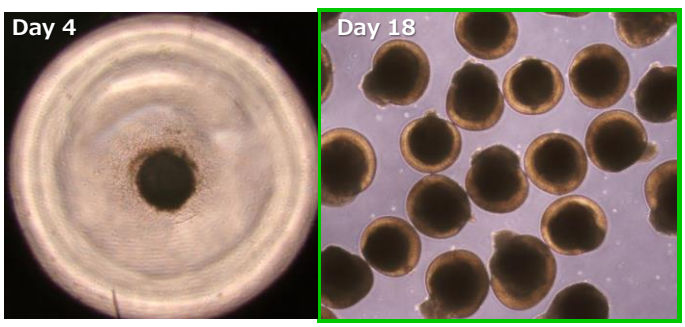


Differentiated Purkinje cells

【 Cerebrum 】



【 Retina 】



FAQ

Q1: How to control the size of the spheroids?
A1: The diameter of the spheroids can be controlled by changing the initial seeding density.

Q2: What is the difference between PrimeSurface flat dish and culture plate?
A2: PrimeSurface flat dishes (35, 60 and 90mm) are flat bottoms and randomly generate multiple spheroids with different size whereas PrimeSurface culture plates (96 and 384 wells) were designed to generate single and uniform sized spheroids in every well.

Q3: What imagers have been used with PrimSurface plates?
A3: Olympus IX71 and Carl Zeiss LSM710
Cell3iMager by Screen Holdings
IncuCyte® by Sartorius
Cytation by Biotek (Now Agilent Technologies)
Celligo by Nexcelom
Opera Phenix by Perkin Elmer

Q4: What are the dimensions of the plate for automation programing?
A4: You can find the dimensions of PrimSurface plate from website. PrimeSurface 96 slit well plate and 384 plate adhere to the standard ANSI/SBS footprint dimensions.

Q5: How do I change media when necessary ?
A5: A 50/50 media exchange is recommended. For example, 96-well plate with a 100 µL starting volume, remove 50 µL of spent media from the wells, and replace with 50 µL of fresh media.

Q6: Are there any special note when using the plate?
A6: As the surface of each well is coated with hydrophilic polymer, during seeding, make sure pipet tips do not scratch the bottom or sides of the wells. Cells may attach to the well if there is a scratch.

Q7: Can I use a centrifuge to spin down the cells?
A7: Centrifugal force of 3000g was used in our internal data but this is highly variable and depends on the cell type and not a guaranteed value.

Reference

+ About 3D culture

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+ About PrimeSurface plates

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