Thank you for purchasing CellHD-256
Please read this handbook carefully before operating the chip

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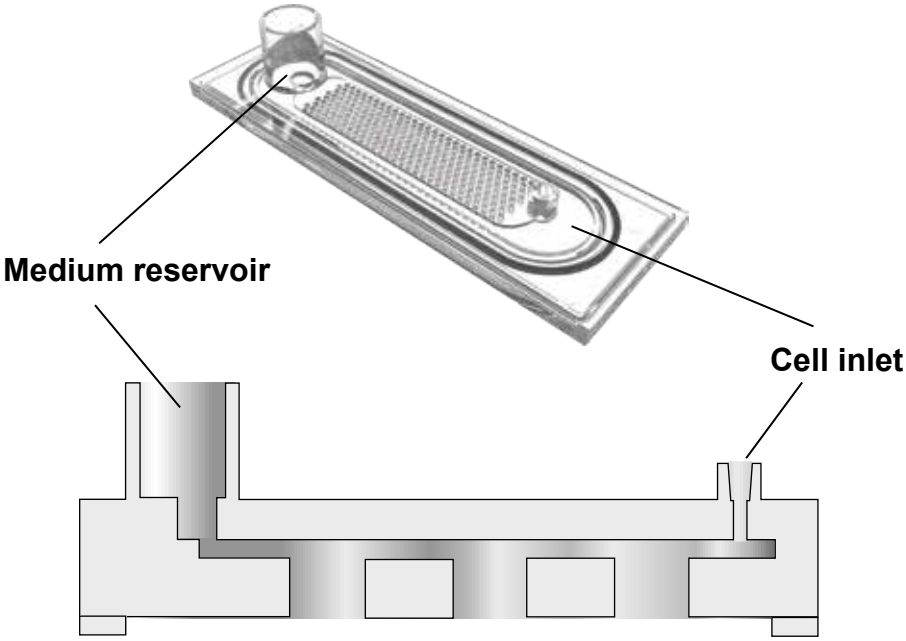
INTRODUCTION | What's in the package?

REMINDERS

★ Please store in a cool, dry place, away from heat and direct sunlight.

★ All components are sterilized. Please Don't use the chip if any damage appears.

★ Open the package **right before using it ONLY.**



PRINCIPLE

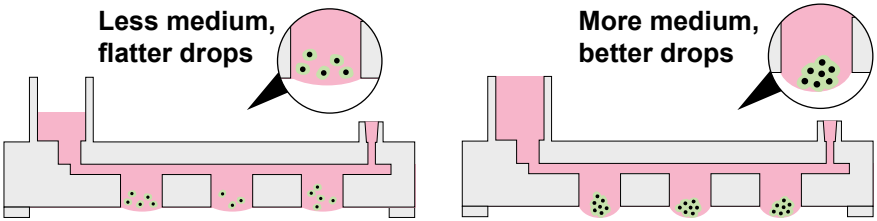
1

CellHD-256 utilizes the **surface tension** to form drops that cells can aggregate into spheroids. The strength of surface tension is an important factor that affects the result of spheroid cells. **Hydraulic differences due to the volume of the medium** is the key factor to affect the strength of surface tension.

CellHD-256 is a 3D hanging drop spheroid culture chip that provides a high throughput, easy use, and low medium for the 3D culture process.

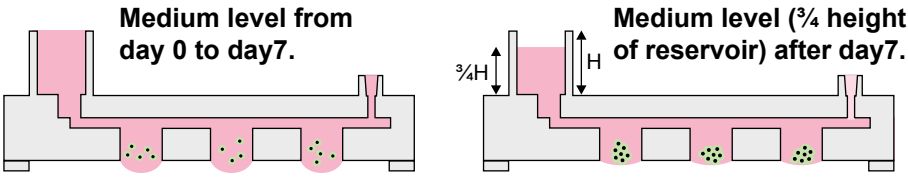
2

The volumes of the medium determine the hydraulic differences. The more medium you add, the better shape of the drop will be formed.



3

We recommend that the reservoir is fully filled with medium from day 0 to day 7, and reduce the medium level to ¾ height of the reservoir to avoid the risk of bursting after day 7.



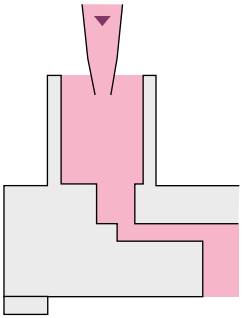
★ **NOTE:**
0.1% of Methyl Cellulose is a common supplement used in 3D culture that helps cells to form spheroids more quickly and efficiently.
Not all of the cells need methyl cellulose to form spheroids. Please check if your cell needs methyl cellulose before culturing.

PREPARE

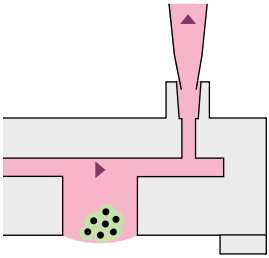
1. Pipet
2. P1000 pipet tips
3. Cell Samples: **750µL** of fully suspended cell is needed.
4. Cell Concentration: Depends on your cell type
5. Medium: Depends on your cell type.
6. Optional Supplement: 0.1% of methyl cellulose.

PRECAUTION

▶ Pipetting Method



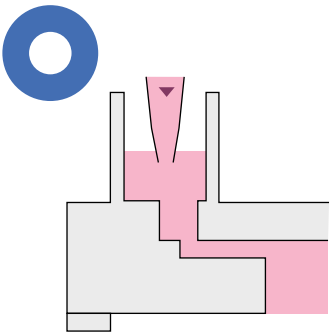
Insert tip around culture reservoir

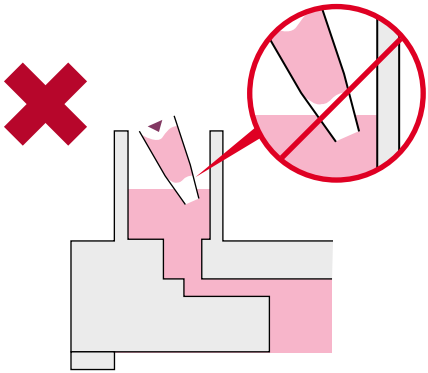


Aspirate from the inlet

▶ Avoid Bubbles

Take care to avoid bubbles in the pipet tip.



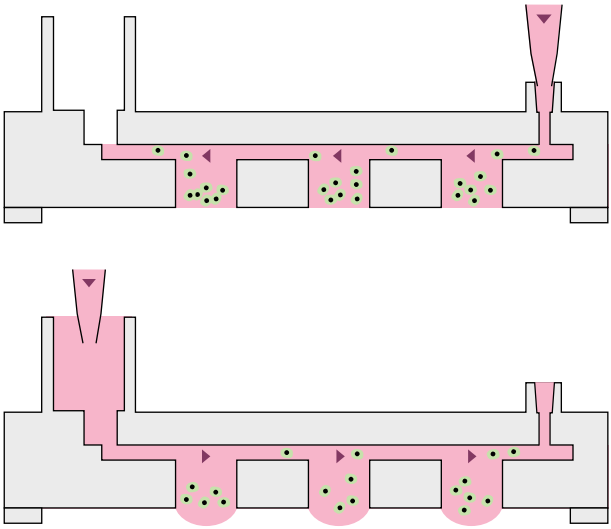


PROCEDURES

Part Ⅰ: Load cells

Step1.
Load **750μL** of cell line slowly from the inlet.

Step2.
Add **450μL** of medium and culture directly if it is not necessary to wash out cells in the channel.



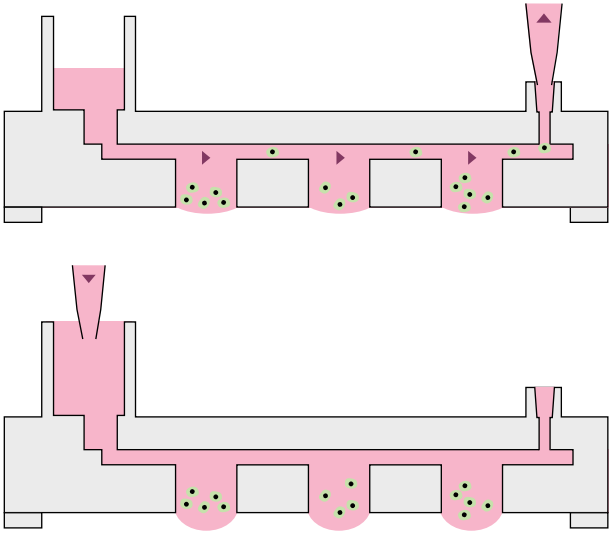
Part Ⅱ: (Optional) Wash out cells

Step1.
Draw out 300μL from the inlet.

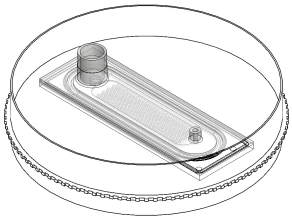
Step2.
Load 300μL from the reservoir.

Step3.
Draw out 300μL from the inlet again.

Step4.
Load medium to fully fill the reservoir.



Part Ⅲ : Culture spheroid cells



Step1. Place the Chip in the petri dish.

Step2. Add 3.5ml PBS to the dish.
(The volume of PBS added each time should not exceed 3.5ml to avoid bursting of 3D beads.)

Step3. Put the dish into the incubator and start culturing.

★ **NOTE:**
If the PBS and medium are evaporated, please add PBS and medium when replacing medium.

Part Ⅳ : Replace medium

Before day7

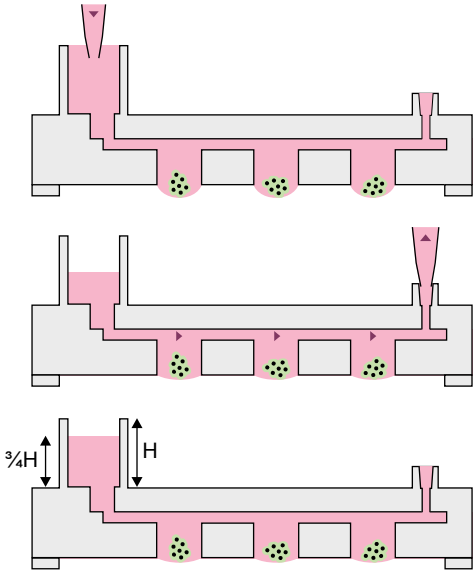
Step1.
Add fresh medium to fully fill the reservoir.

Step2.
Draw out 200μL from the Inlet slowly.

Step3.
Repeat Step1 and Step2 **twice** and ensure the reservoir is fully filled to complete the process of replacing the medium.

After day7

Repeat the same steps above.
Notice that the upper limit of medium is ¾ height of the reservoir to reduce the risk of bursting.



★ **NOTE:**
Drawing out medium slowly helps to keep spheroids inside the drops. Reducing the medium (3/4 height of the reservoir is recommended) helps to prevent from bursting.

Part Ⅴ : Harvest spheroid cells

Step1. Prepare a new dish

Step2. Add 1 mL medium to the central area.

Step3. Put CellHD-256 over the medium in the dish.
The drops will burst when contacting the medium.

Step4. Use a pipet to harvest the spheroid cells.

★ **NOTE:**
Be careful!! Do NOT touch the medium after the drops burst.

FAQ:

1. Q: What is the principle used by CellHD-256?

A: CellHD-256 utilizes surface tension due to the hydraulic difference to form droplets that sediment cells to aggregate spheroids. The higher hydraulic difference forms better droplets that help to create uniform circularities and spheroids of cells.

2. Q: Why do the drops burst?

A: CellHD-256 utilizes the surface tension to keep the shape of the droplet. Once the surface tension can not hold the drops due to a critical hydraulic difference (occurs after culturing for several days) or shaking/vibrating of the chip when refreshing the medium or moving the chip. The drops will burst.

3. Q: How can I prevent drops from bursting?

A: There are several ways to reduce the risk of bursting.

1.Reducing the medium after day 7.

2.Replacing the medium gently.

3.Keeping CellHD-256 in a static state as you can.

4. Q: How many days can I keep the drops?

A: Generally, the drops can be kept for 7 days. It varies due to the different cells.

Some cells may last more than 7 days.

5. Q: Is washing out cells a necessary process of CellHD-256?

A: No. You can culture the cells directly without washing out cells in the channel. Washing out residual cells in the channel helps to reduce unnecessary waste of the medium and increase the cycle of replacing the medium.

6. Q: Is there any other way to improve spheroid forming rate?

A: Yes. From our experience, adding **0.1% of methyl cellulose** helps to improve the spheroid forming rate.

7. Q: What is the functionality of the transparent film?

A: This is a hydrophilic film that helps to observe the spheroid cells after taking the chip from the incubator.

Please keep ORIGEM face up when placing CellHD-256 on it.

ORIGEM

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