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

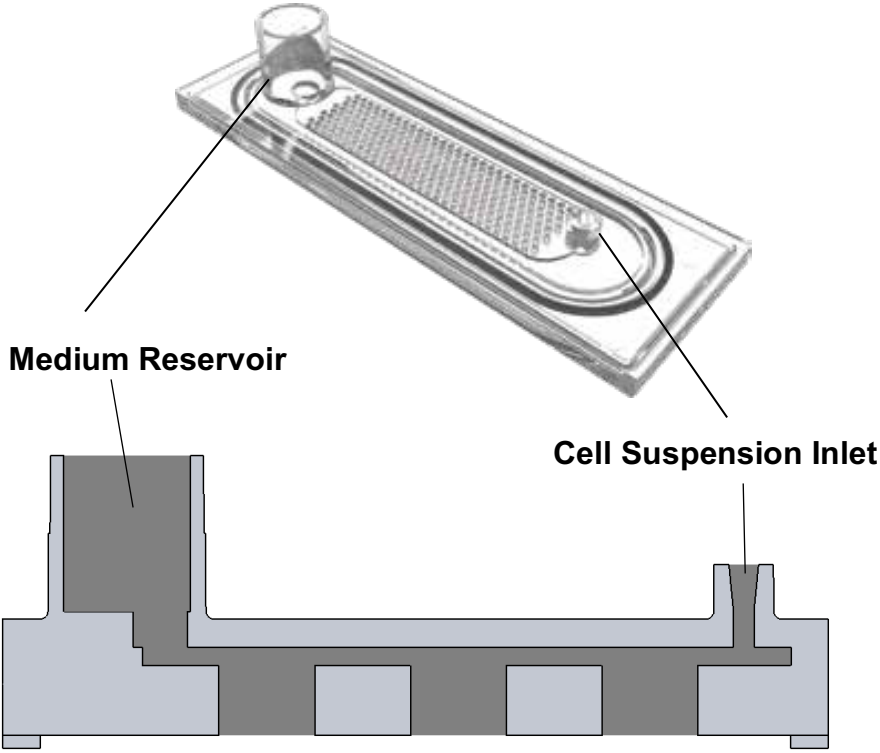
CONTENTS

INTRODUCTION | What's in the package?

Note

★ The package should be stored in a dry place at room temperature.

★ All components inside the package have been sterilized.

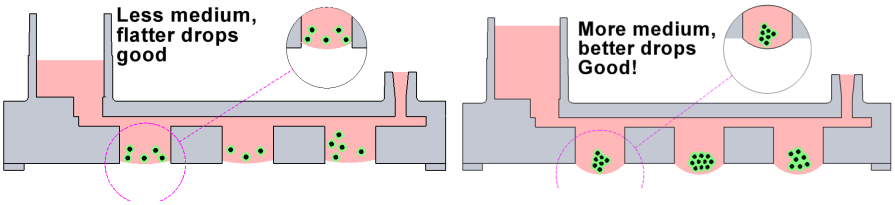


PRINCIPLE

1

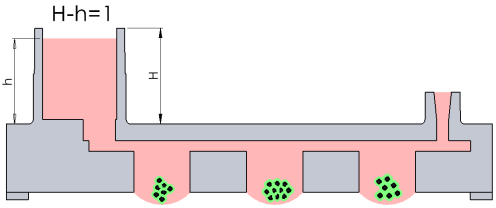
CellHD-256 utilizes open holes in the bottom of a microchannel to form medium drops in which suspended cells can aggregate at the bottom of each drop. The concave shape of the drops is needed for forming a single cell spheroid in each drop, and is controlled by the medium height in medium reservoir; the higher the liquid level in the reservoir the more curvature the drop has.

2



3

We recommend filling the reservoir with medium up to near the top of the reservoir (~ 1 mm away from the top)



★ **NOTE:**

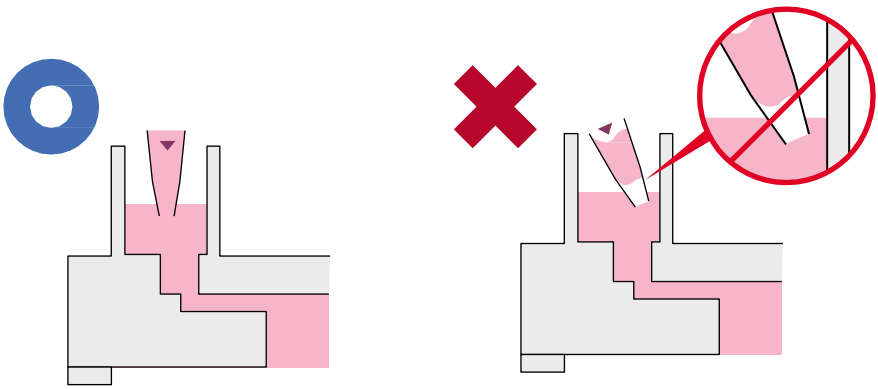
Methyl Cellulose is a common supplement added in the medium (e.g. at 0.1% g/mL concentration) to facilitate 3D cell spheroid formation. Some cell types do not need methyl cellulose to form spheroids.

PREPARATION

1. 10 cm Petri dish: 1 dish is needed for one chip.
2. P1000 and P200 Micropipette
3. P1000 and P2 pipet tips
4. Cell Suspension: 730µL is needed for one chip. The cell Concentration depends on your cell type.
5. Medium: 730µL is needed for one chip.
6. Optional Supplement: 0.1% g/mL methyl cellulose in the medium
7. 1xPBS buffer solution: 4.5 mL is need for one chip.

PRECAUTION

- **Avoid air bubbles formation when adding medium to the reservoir.**
  Make sure the pipet tip does not have an air pocket and should be submerged under the air-liquid interface before pushing out the medium to the reservoir.



PROCEDURES

Part Ⅰ: Forming cell spheroids

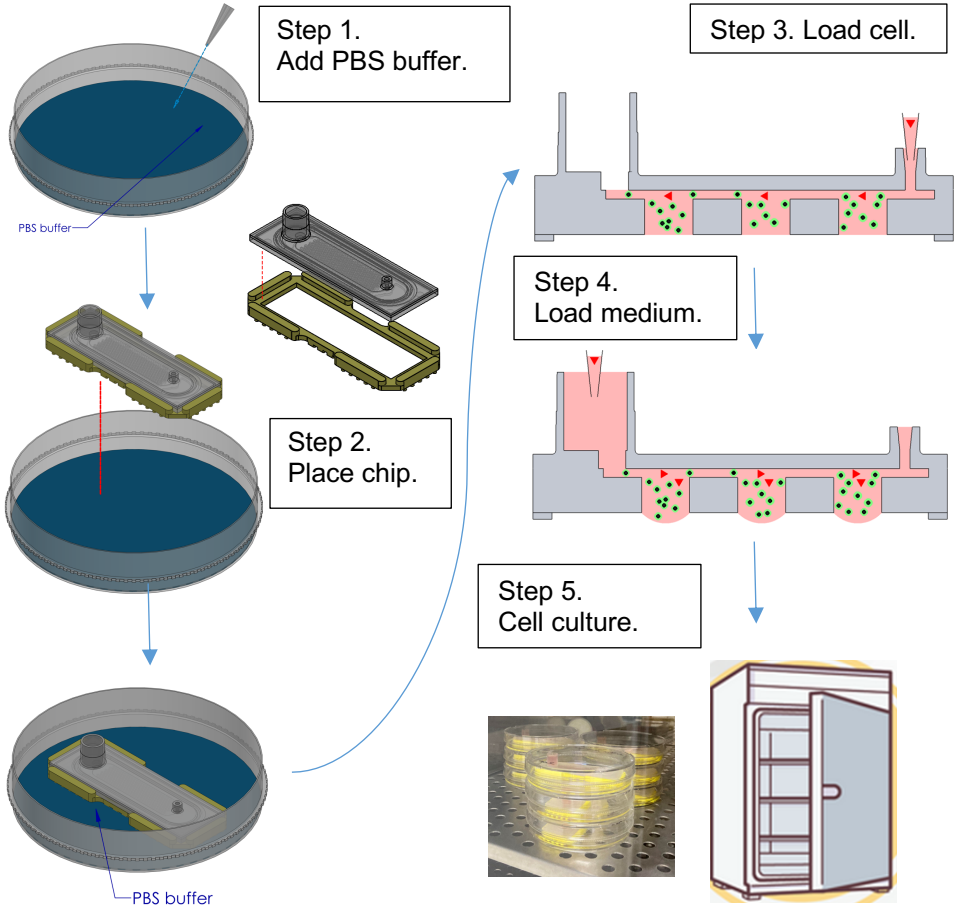
Step1. Add 4.5 mL of 1x PBS buffer solution to a 10 cm Petri dish.

Step2. Place the chip on the chip stand and then place them inside the Petri dish.

Step3. Load a pipet tip with 730 μL of cell suspension, insert it in the cell suspension inlet and gently push out 710 μL of the cell suspension from the tip (Do NOT push all the cell suspension out from the pipet tip to avoid injecting air bubble into the microchannel). Wait for 10 seconds, and then gently remove the pipet tip from the inlet hole.

Step4. Gently add 400μL of medium to the reservoir.

Step5. Cover the Petri dish with its lid and place it the inside a cell culture incubator.



Part Ⅱ: Medium exchange

★ **NOTE:**

The medium should be exchanged every 2-3 days, or according to your specific experimental design。

Step 1. Inspect the Cell Suspension Inlet: If the liquid level is low add some medium to fill it up. If an air bubble is trapped in the inlet (see Figure 1), remove the air bubble with a pipet tip before filling up the inlet hole with medium.

Step 2. Use a 200 μL pipet tip to aspirate 200 μL of medium from the reservoir.

Step 3. Add some medium to the suspension inlet hole to fill it up. Load a 200 μL. pipet with 200 μL of medium, insert the tip into the cell suspension inlet hole and inject the medium to the microchannel.

Avoid introducing air bubble to the microchannel; the pipet tip should be. submerged under the air-liquid interface and not air pocket is trapped in the pipet tip.

Step 4. Repeat step 2-3 for 5 times.

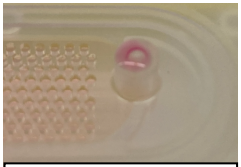
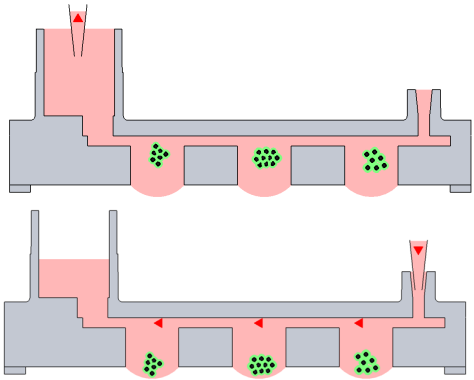


Figure 1. Picture of a trapped air bubble at the inlet



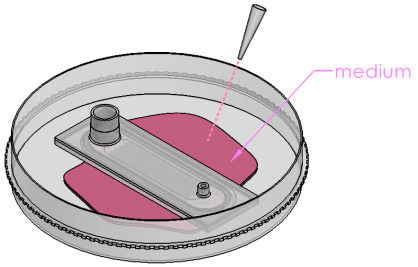
Part Ⅲ : Harvesting Spheroids

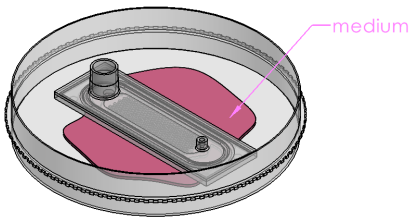
Step 1. Prepare a new 10 cm Petri dish.

Step 2. Add 1 mL of medium to the central area of the dish.

Step 3. Put CellHD-256 on top the medium in the dish to burst the drops into the medium.

Step 4. Use a pipet tip to harvest the cell spheroids in the medium.





FAQ:

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

A: CellHD-256 utilizes a patented microchannel design to high-throughputly generate hanging drops in which uniform cell aggregates can formed and grow into 3D cell spheroids on chip.

2. Q: How to prevent drops bursting?

A: 1. Operate gently when during medium exchange. 2. Handling the device without abrupt movement. 3. Do not tilt the Petri dish to prevent drops touching the PBS solution in the dish.

3. Q: What cell suspension concentration should be used for seeding?

A: The cell suspension concentration determines the initial cell spheroid. size, and should be decided based on cell type and experimental design. We suggest a cell suspension concentration range of 1~10 x 10^4 cell/mL to start with.

4. Q: What to do if the cells do not form spheroid in the drop?

A: Adding methyl cellulose to the culture medium (at 0.1% g/ml) may help spheroid formation.

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