ORIGEM

Thank you for purchasing CellGem™.

Please read this handbook carefully before operating the chip.

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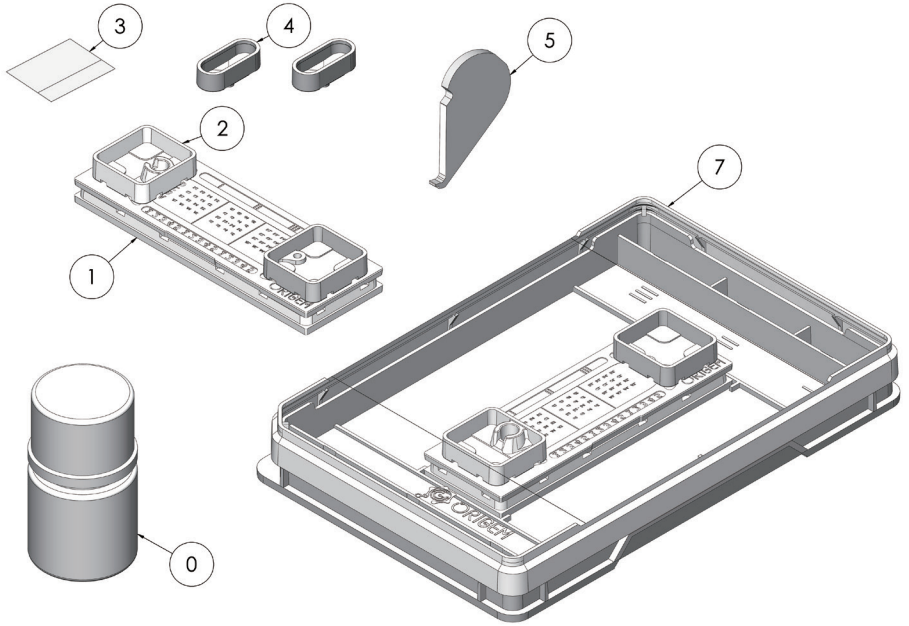


⓪ Priming solution
① CellGem™ chip
(Already connected to ② reservoir)
② Reservoirs
③ Sealing tape
④ Cell culture reservoirs
⑤ Disassembly tool
⑥ Tweezer
⑦ Chip carrier
⑧ Operation handbook and card for recording data

REMINDERS

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

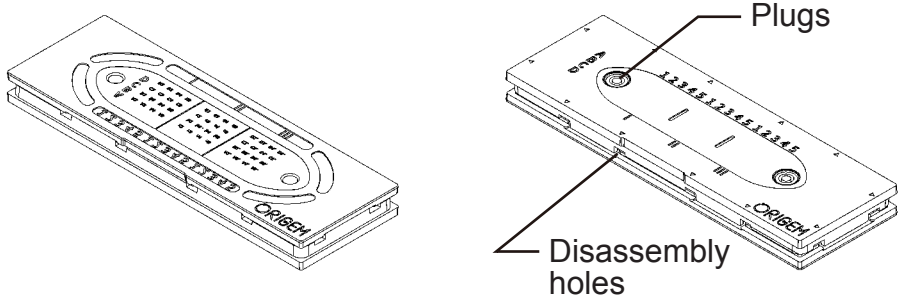
★ All components are sterile packaged. Please Don't use if any packages are torn or damaged.

★ Only open the package containing the cell culture chip **right before use.**

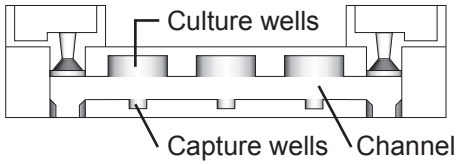
THE CellGem™ CHIP

Cell **capture** side
(Starting Side)

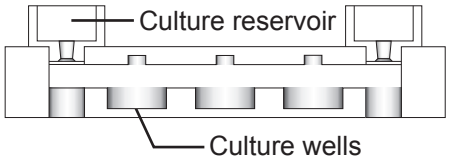
Cell **culture** side



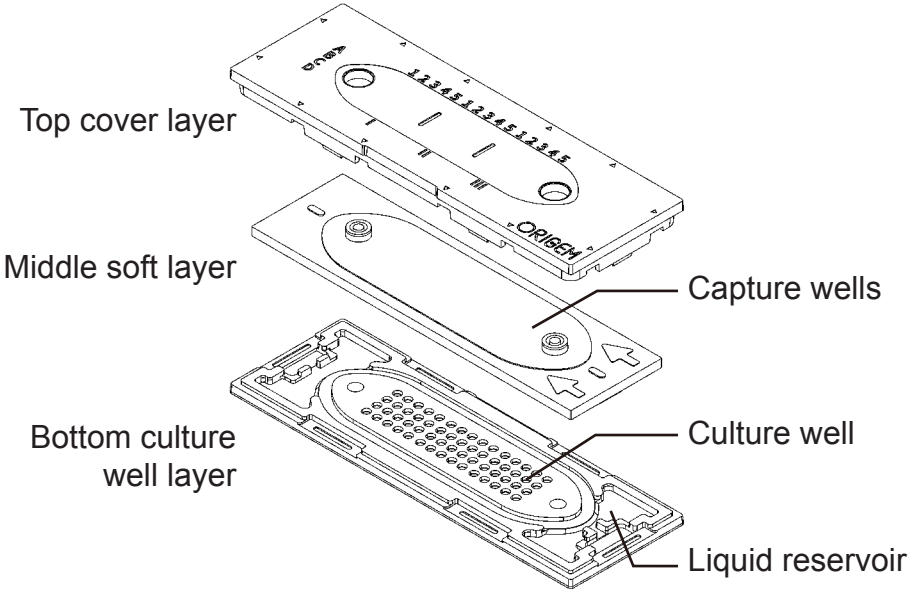
Cell capture wells facing up



Cell culture wells facing up

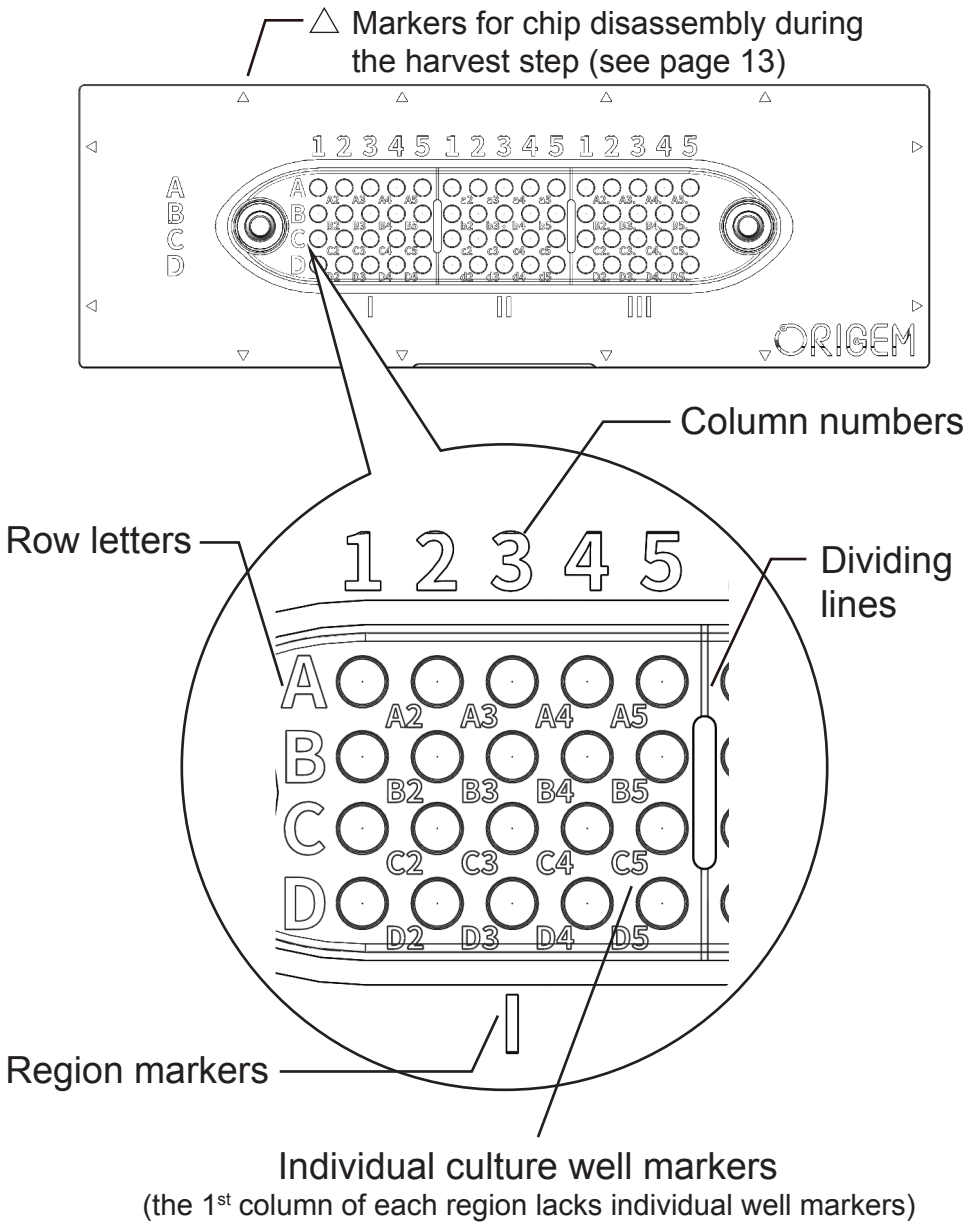


The CellGem™ chip is comprised of 3 different layers which are disassembled during the harvest step (see page 13).



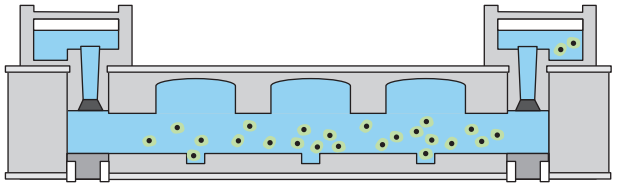
MARKERS ON THE CHIP

The culture wells are divided into 3 regions on the chip,
each region containing 5 x 4 = 20 wells, adding up to a total of 60 wells.
The 3 regions are separated by dividing lines.

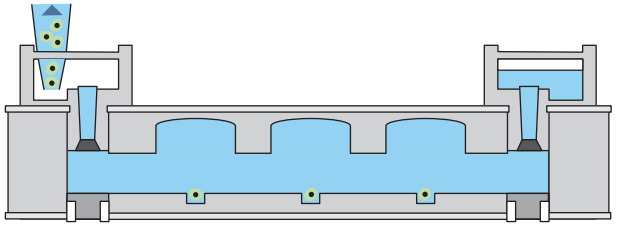


PRINCIPLE

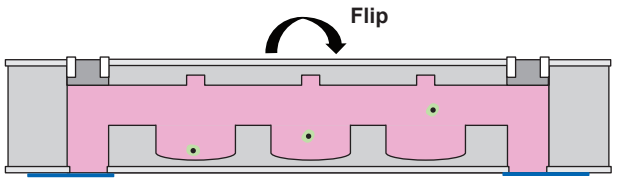
Cell loading



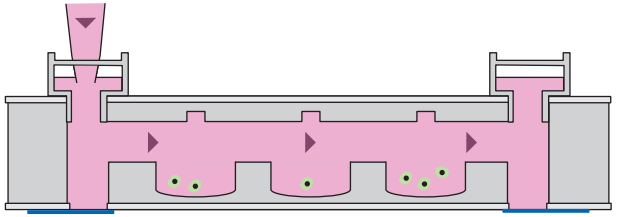
Washing out uncaptured cells



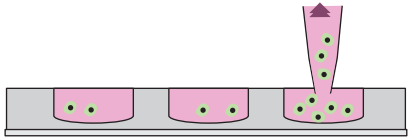
Seal and Flip



Culture



Harvest

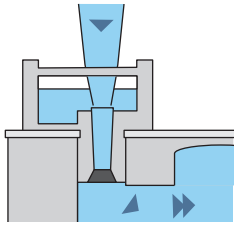


PREPARE

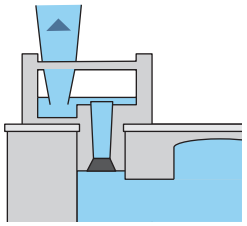
1. Pipet
2. P1000 pipet tips
3. 3 or 5 mL syringe
4. Tweezer
5. Inverted microscope
6. Centrifuge with microplate-compatible swing bucket

PRECAUTIONS

▶ **Pipetting Method**

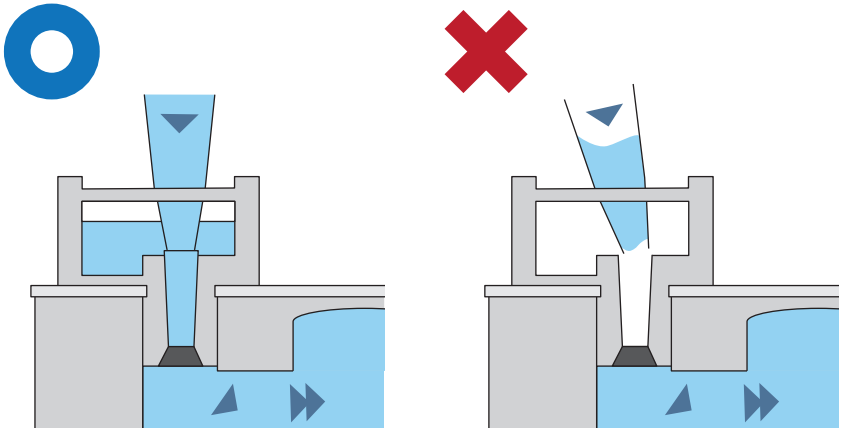


Dispensing
Insert tip into inlet port



Aspirating
Aspirate **around** the inlet port

▶ **Avoid Bubbles**
Take care to avoid bubbles in the pipet tip and make sure reservoir inlets are filled with liquid.



II PREPARATION OF CELL SAMPLES

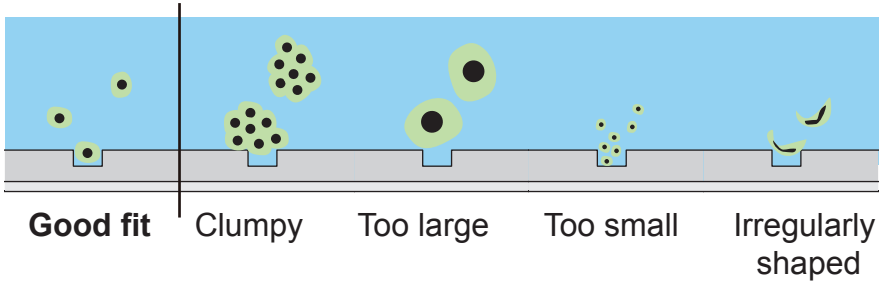
▶ **Use similar practices as preparing cell samples for flow cytometry. Preventing cell clusters is key.**

▶ Prepare your cell sample with a concentration of $5x10^5$ to $1x10^7$ cells/mL (the specific concentration may be adjusted depending on experimental conditions)
A recommended starting concentration is $2x10^6$ cells/mL.

▶ Fully resuspend your cells in solution.
Each chip would need 600 µL of cell suspension.

▶ Recommended solutions for cell resuspension.
-1X PBS
-Low serum/serum free medium
-FACS sorting buffer
-Cell dissociation buffer

▶ Cell morphologies can affect capture efficiency.



CellGem™ Specs	Suitable Cell Size (Diameter)
S	8-12 µm
M	11-17 µm
L	14-25 µm

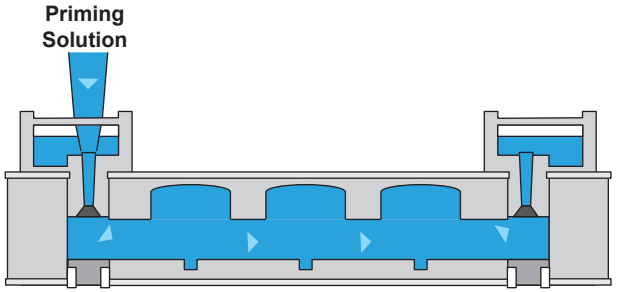
III PROCEDURE (3 MAJOR STEPS)

Cell capture ➡ Cell culture ➡ Cell harvest

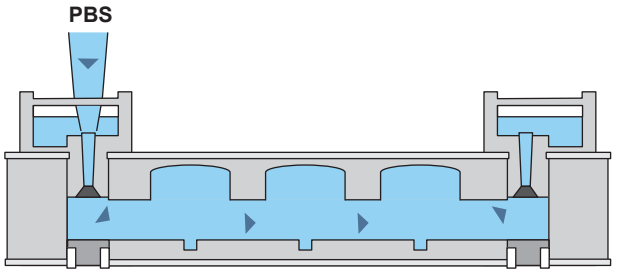
Part I : Capture CELL CAPTURE

Pre-rinse

1 Slowly inject 1 mL of priming solution into either inlet port of the CellGem™ chip using a P1000 pipet and let sit for 10-30 seconds. Make sure the entire chip is filled with solution and free of bubbles. If bubbles are present, aspirate out all the solution and start over again. (See detailed operation in video).



2 Replace priming solution in the chip with PBS: inject 1 mL of PBS into the chip using P1000 pipet to displace priming solution, aspirate out the waste solution from the other port, and repeat 3 times.

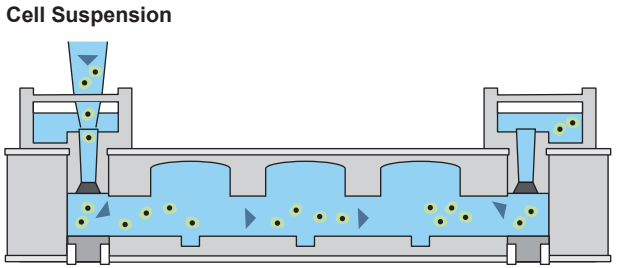


QUICK TIP

★ When injecting any solution, make sure the very tip of the pipet tip or syringe does not contain air bubbles. Air bubbles can significantly affect the performance of the chip.

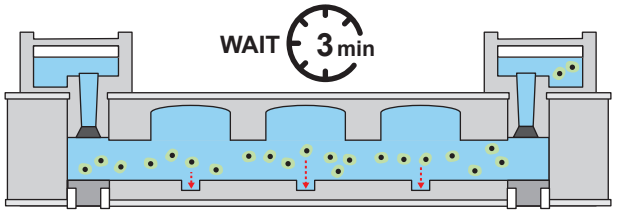
Cell loading

3 Inject 500-600 µL of the prepared cell suspension into the chip (recommended starting concentration: $2x10^6$ cells/mL, adjustable depending on experimental conditions). Wait 3 minutes to allow cells to settle to the bottom of the chip and get captured by the capture wells.

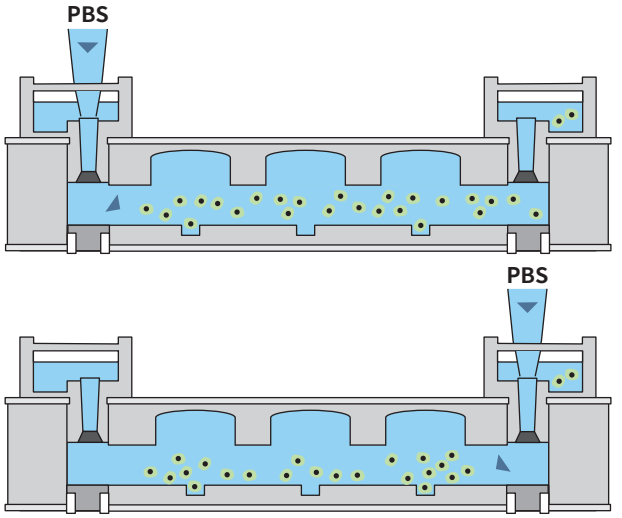


QUICK TIP

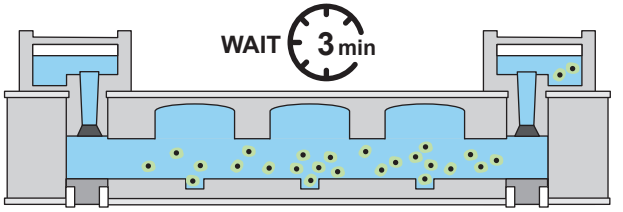
★ **Make sure to fully resuspend cells in solution before injecting into the chip.**



4 (Optional) After the 3-minute wait, inject 100 µL of PBS to both inlet ports (sequentially) to help “nudge” the cells into the capture wells to further increase capture efficiency. Wait another 3 minutes.

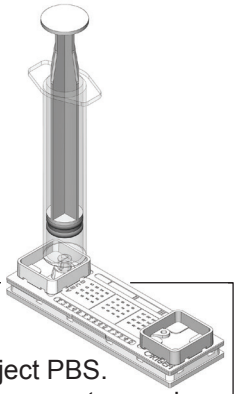


5 Check the state of cell capture under a microscope. If needed (i.e. lots of empty wells remain, cells not well distributed), repeat step 3 or step 4.



Washing out uncaptured cells

6 (Optional) Wash out uncaptured cells using 1 mL PBS.
(The washed-out cells can be collected and reused).



7 **Use a syringe or pipet TIP to wash channels:**

7.1 Draw a 3 mL or 5 mL syringe full of PBS, insert the syringe into the reservoir, and push plunger to inject PBS. At the same time, use a pipet to aspirate out the waste wash solution.

7.2 When syringe is nearly depleted (with a small amount of PBS left), fully aspirate all the liquid in the reservoir using a pipet. (Do not remove syringe yet).

7.3 Wait 3 minutes to allow cells to settle for better washing performance. Add fresh PBS into the reservoir to fully cover inlet ports, then remove syringe (this prevents air from being introduced into the chip during syringe removal).

7.4 Repeat the above steps 2~4 times, alternating between both inlet ports, ensuring no non-captured cells remain.

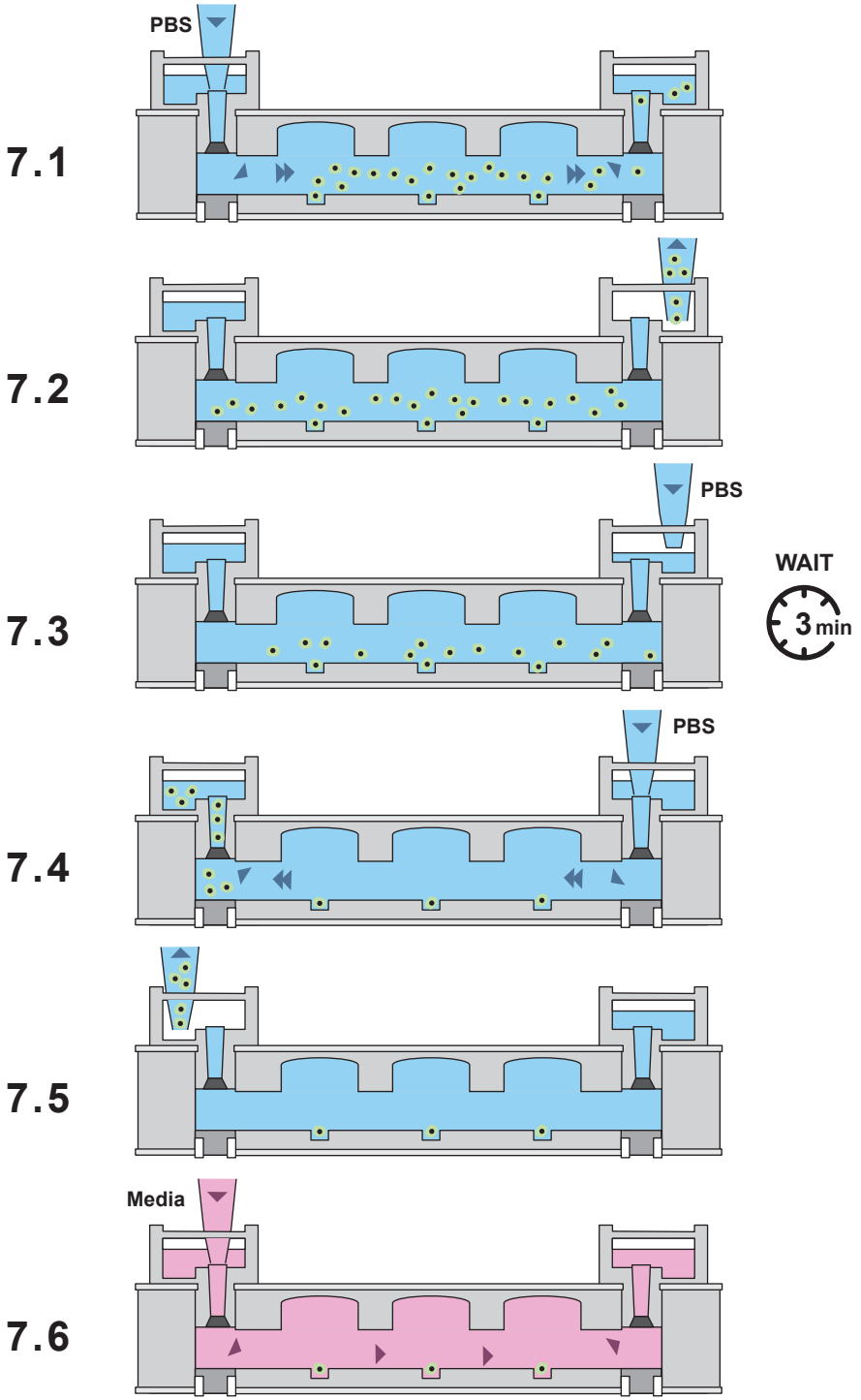
7.5 Inspect the captured cells under a microscope. If capture efficiency is not ideal, one can start again from step 3 (cell loading).

7.6 Replace the PBS in the chip with cell culture media. Inject 1~2 mL of media into both inlet ports using a pipet.

QUICK TIP

★ There is no limit on how many times you can wash the chip. The goal is to fully remove residual non-captured cells from the chip, to prevent satellite colonies from growing in unintended places in the chip.

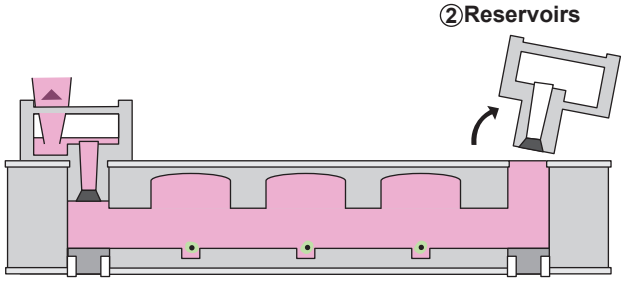
★ Multiple media exchanges can be done to ensure complete PBS displacement.

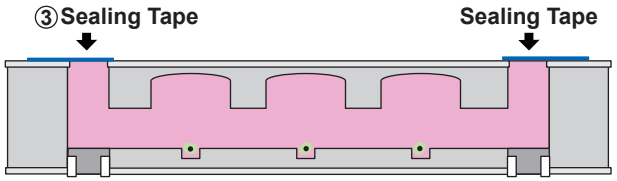


Seal and Flip

8

Fully aspirate all the remaining liquid in the reservoirs, then remove the reservoirs from the chip. Wipe off any liquid on the surface of the chip, then fill up the inlet ports with media. Seal off the inlets with the included sealing tape.

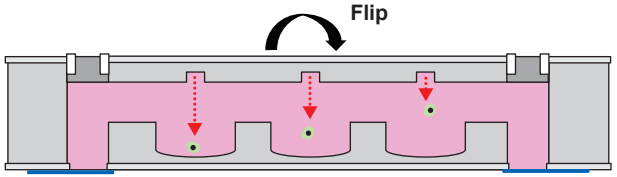




(The tape is pressure sensitive, so make sure to apply pressure to fully seal the chip).

9

Flip the chip over 180º, and let sit for 20 min to allow cells to settle naturally into the culture wells. Alternatively, you can place the chip into the chip carrier⑦and spin the cells down in a centrifuge (100 G, 2 min). Add 2 mL of 1X PBS into both reservoirs in the chip carrier to maintain culture humidity, and cover with lid. Record single cell locations using the included cards, and place the chip with chip carrier into a cell culture incubator.



QUICK TIP

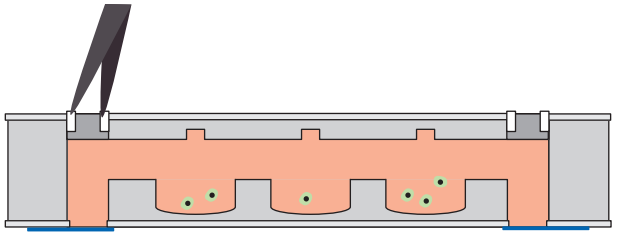
- ★ Stickers should not cover/overlap with the position of the culture well, which can affect observation.
- ★ Make sure the centrifuge is balanced.
- ★ Do not centrifuge at higher speeds, as single cells can escape from the wells.

CELL CULTURE

Install cell culture reservoirs for changing media

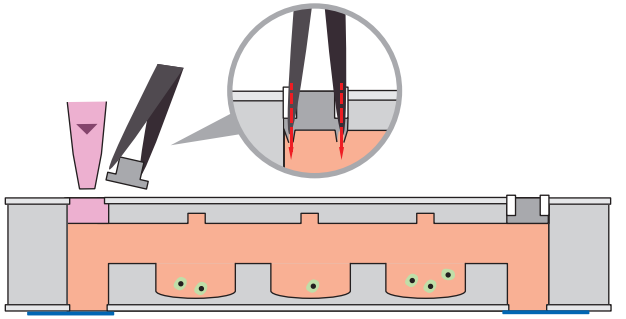
1

After culturing for 1-2 days (after cells have fully attached), install cell culture reservoirs ④.



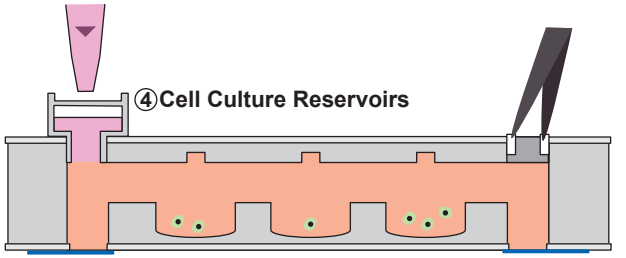
2

Use tweezers⑥to remove plugs. **Apply pressure to break the weak attachment before pulling out the plug. Make sure to not puncture the bottom sealing tape by inserting the tweezer too deep.**



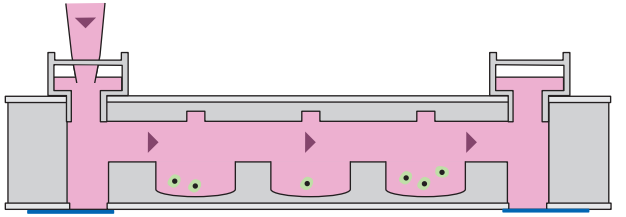
3

Fill the ports with media, then install cell culture reservoirs ④ onto the chip. *Slowly* inject fresh media into the chip.



4

Refresh media every 2-3 days. Adding fresh media into one of the reservoirs will slowly refresh the media in the chip via hydrostatic pressure difference.



QUICK TIP

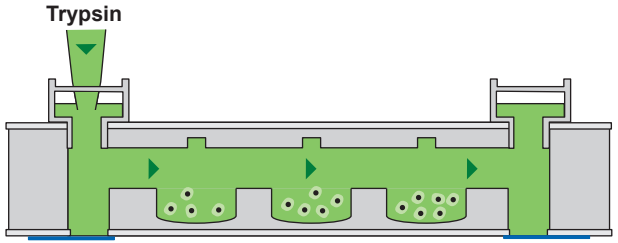
- ★ If there is any small bubble in the chip, please slowly inject media into the chip in order to remove bubble out.
- ★ If there is no hydrostatic pressure difference, please slowly inject media into the chip to remove any bubble around the inlet ports.

Part III : Harvest

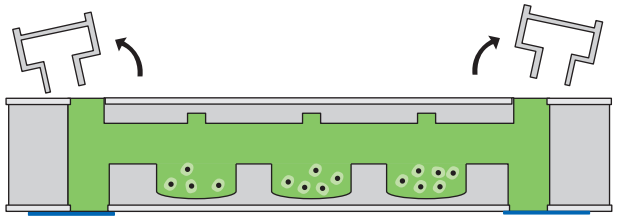
CELL HARVEST

After culturing for 7-14 days, disassemble the chip for harvesting cells. (Exact culture time will depend on cell type).

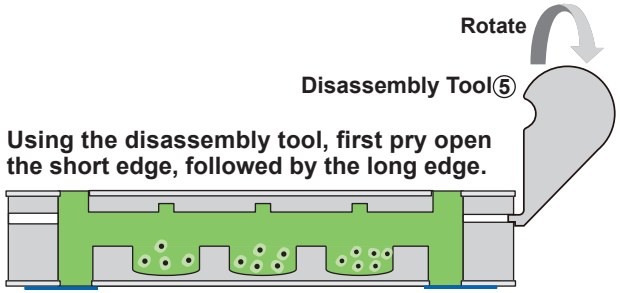
1 Replace cell culture media with PBS, followed by replacing the PBS with Trypsin. Incubate in incubator at 37 ºC for 3 min.



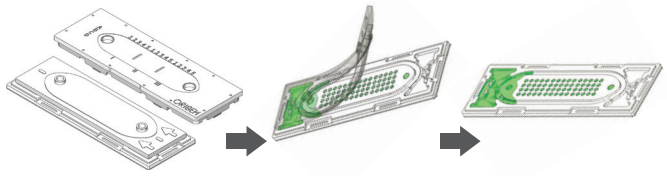
2 Before cells become suspended, remove the cell culture reservoirs.



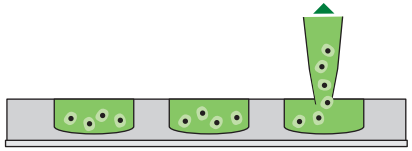
3 Insert disassembly tool into the disassembly holes on the side of the chip (see page 3 location markers). **Rotate** the disassembly tool to pry open the chip.



4 After prying open the chip, remove the top (hard) cover, slightly tilt the chip and softly **peel up** the middle soft layer, allowing the liquid to pool in the liquid reservoir on the side of the chip or outside the chip.



5 Use a 2 µL pipet to aspirate the cells from the culture wells and transfer them to a larger culture plate. **Each culture well has a working volume of 1.5 µL.**



IV Q&A

1. Q: An air bubble was trapped in the chip during the priming step. How do I get rid of it?

A: Fully aspirate the priming solution from the chip, then rapidly inject the priming solution back into the chip. This should remove any bubbles.

2. Q: Is there a contamination risk with using sealing tape to seal the chip inlets?

A: The sealing tape used in the kit is tested and certified to be sterile. It's safe to use with cell culture.

3. Q: After flipping over the chip, how do the captured cells accurately land in their corresponding culture wells?

A: The capture wells are specifically designed to ensure that each captured single cell will only land in its designated culture well after flipping the chip. However, the chip can only be flipped over once. **Users should avoid flipping it over again after the single cell transfer step.**

4. Q: What's the purpose of centrifuging the chip?

A: This accelerates the transfer of captured single cells into the culture wells. However, users should avoid centrifuging at high speeds to avoid cell loss (we recommend centrifuging at 100 g for 2 minutes).

5. Q: What are the advantages of CellGem™ compared to other single cell separation products on the market?

A: Our product does not require complex instrumentation and is easy to use. It also boasts high single cell capture efficiency, high single cell growth rate, and consumes minimal cell culture media.

6. Q: Following the previous question, what do you mean by high single cell growth rate? In theory single cells don't grow into colonies very efficiently, why is this?

A: The main reason is because all the cells inside the CellGem™ chip share the same pool of cell culture media, and hence, also share secreted growth factors between each other, all the while still allowing each single cell to have its own isolated growth space. (Depend on cell type.)

7. Q: Why is my cell capture efficiency low? How do I increase single cell capture efficiency?

A:
1. If your cells are clumpy, then they cannot be efficiently captured by the capture wells. We recommend trying a cell dissociation reagent that's compatible with your cells (ex. Accumax, sorting buffer.)
2. There may be excess bubbles in the chip, affecting cell capture efficiency. We recommend trying the bubble removal method in page 8 of the handbook.(Page 7)
3. You may have selected a capture well size that's not a good fit for your cell of interest. We recommend measuring the size (diameter) of your cells and selecting the product with the corresponding suitable capture well size.(Page 6)
4. Your cell loading concentration may be too low. If so, we recommend performing the cell loading operation multiple times, or concentrate your cell suspension before loading.

8. Q: If there are cells present in the channel, will it affect the monoclonality of my single cell-derived colony? If so, how do I perform the subsequent cell harvesting steps to avoid contaminating my single cell colonies?

A: Cells present on the channels do have the potential of contaminating your single cell colonies and affecting monoclonality. So It's important to ensure that the washing step is done well to remove excess cells from the chip. If you still have residual cells left in the channel, then during the cell harvesting step, do not directly inject trypsin into the chip. Instead, disassemble the chip while the cells are still attached, then individually add trypsin to each of the culture wells to detach the cells. **Each culture well has a working volume of 1.5 µL.**

ORIGEM

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