For life science research use only.
Not for use in diagnostic or clinical procedures.

Updated June 9, 2021

TASCL™ 1000 Well and TASCL™ 600 Well

Product No. TASCL1000 and TASCL600

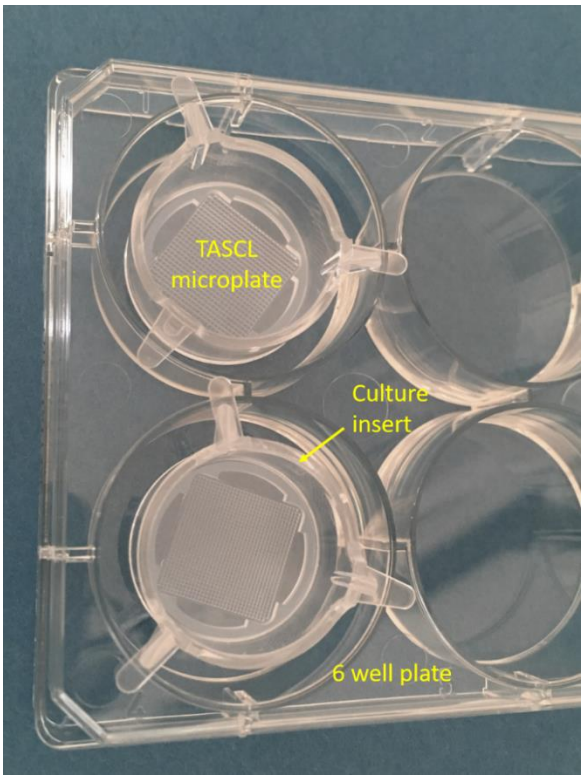
****Store at Room Temperature. Do not Freeze.**

TASCL (Tapered Stencil for Cluster Culture) microplates are provided sterile and ready to use. The TASCL microplate stencil is made of pretreated silicone rubber and seated to the "culture insert", which is placed in each well of the 6-well culture plate. TASCL is good for one year if the package remains sealed. Please refer to the package for the specific expiration date.

Components

- 1x 6-Well Plate with cover
- 6x Culture Insert
- 6x TASCL microplate

TASCL samples are provided without 6-Well plate. 1 TASCL1000 microplate or 1 TASCL600 microplate with 1 Culture Insert in sealed sterilized pouch.

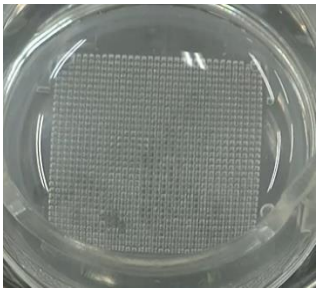


Instruction for Use

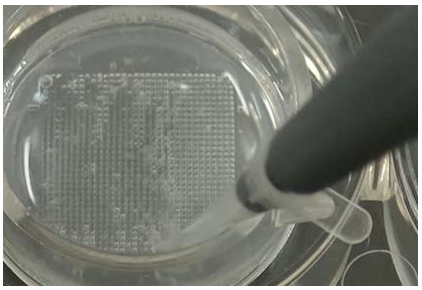
This is the standard protocol for using TASCL microplates. Modifications may be necessary depending on your application.

1. **Preparing TASCL microplate for use – Inspection and removal of air pockets and bubbles**

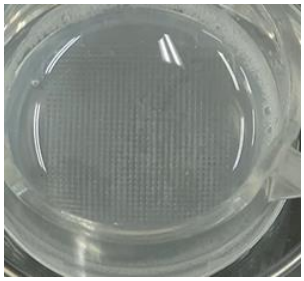
   - Set TASCL microplate with culture insert in the well. Drop 2ml of culture medium in the TASCL microwell, spread to cover all surface of microwell area. Inspect the microplate for trapped air bubbles. Whitish look of the microplate is a sign that aire bubbles are trapped.



Air trapped in ~95% of microwell

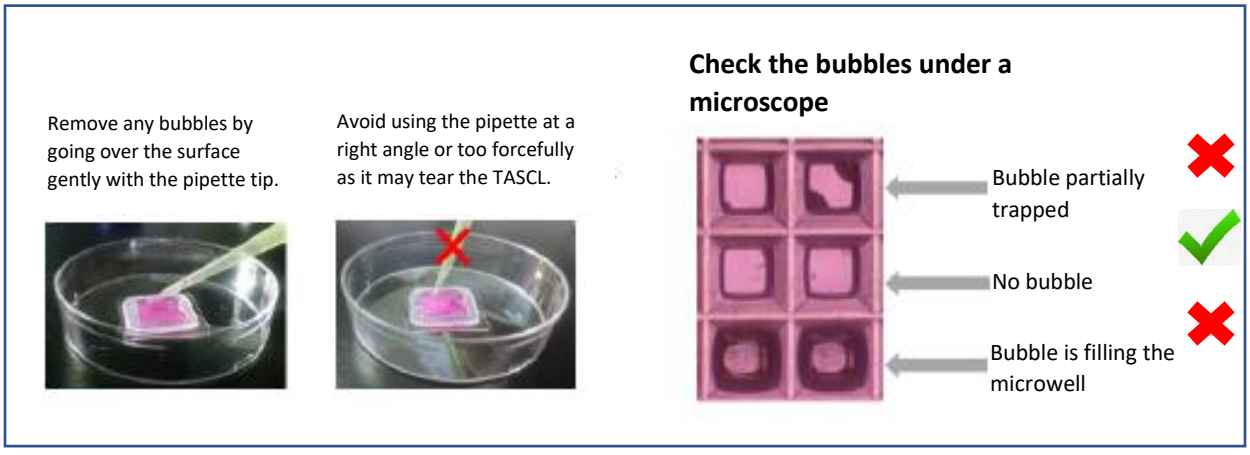


Removing air from microwell with pipette tip



Air completely removed

   - Carefully remove the bubbles from the microwells by gently going over the surface with a 200µL micro pipette tip.



2. **Preconditioning the microplate**

- Keep the medium added in step above in the microplate. Incubate for 10 to 15 min at 37°C while you are preparing the cell suspension.

2. Preparation of cell suspension

The capacity per microplate is 0.5mL. Calculate the necessary number of cells to be seeded per TASCL based on the concentration below.

Cell density and optimal volume may vary with cell types and must be adjusted.

	# of cells in 1mL (concentration)	# of microwells / TASCL	# of cells / microwell *
TASCL1000	~ $1x10^6$ to $6.2x10^6$	1020	~ $0.5x10^3$ to $3x10^3$
TASCL600	~ $2.4x10^6$ to $12.4x10^6$	621	~ $2x10^3$ to $10x10^3$

*The volume of each microwell in TASCL1000 is smaller than in TASCL600. Thus a lower cell density is recommended.

3. Seeding cells

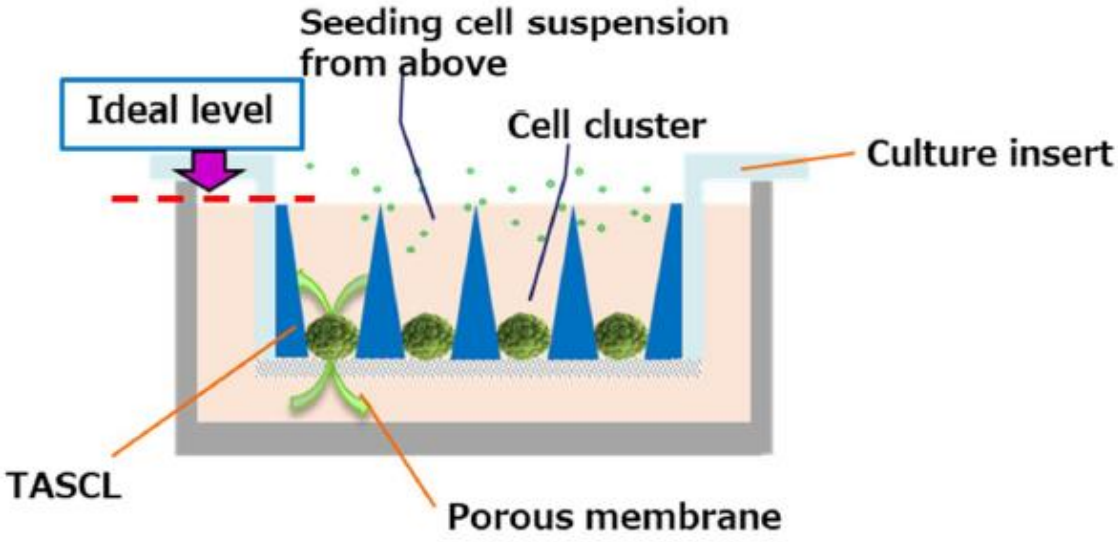
- Aspirate most of the medium out used for incubation in Step 2.

- Carefully dispense 0.5mL of the suspended cells (***3. Preparation of cell suspension***) onto the TASCL microplate over the entire microwells (mesh area). Wait until the cells settle in the microwells.
- With inverted microscope, you can usually start observing the cells in the microwells within 5 min. It will take about 1 hour for all cells settle to the bottom of the microwells.

4. Medium level adjustment

- Add 3.0mL of culture medium between the culture insert and the side of the well.
- Add culture medium between the side of the well and the culture insert to meet the ideal level shown in the figure below.

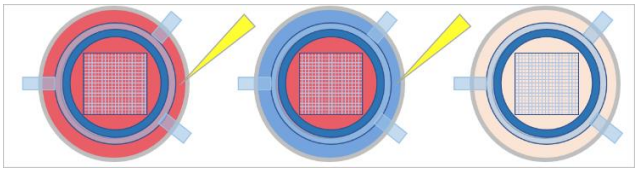
Note: The ideal level of medium should be the same as surface of the microplate (see figure below). Insufficient medium will cause the medium in the microwells to be siphoned out. Too much medium may cause the cells in the microwells to overflow.



5. Refreshing the medium

Method 1

- Pipette medium out from the space between the culture insert and the wall of the well as much as possible. Add fresh medium from the side of the well. Do not pipette or add medium within the culture insert.
- Adjust the medium level as described in ***6. Medium level adjustment*.**



Method 2 (shown in instruction video)

- Move the culture insert with TASCL to the empty well, aspirate the old medium out.
- Add 3mL of fresh medium (~3mm in height), then set back the culture insert gently. Rapid placement of culture insert may cause the cells/spheroids to float out of the microplate.
- Adjust the medium level as described in ***6. Medium level adjustment***.

Notes:

* Medium in the TASCL microplate will gradually exchange through a porous membrane of the culture insert.

* Accumulation of substances in the microwell can block the spontaneous exchange through the membrane. This occurs more commonly when culturing primary cells.

* The frequency of refreshing the medium depends on the cell type used and factors such as the proliferation rate.

* Carefully monitor the change in medium level caused by evaporation. Add medium as needed to maintain the level as described in ***6. Medium level adjustment***.

6. Collecting cell spheroids

- Pipette medium and gently spray the microwells to create overflow. The overflow will release the cell spheroids from the microwells. If needed add about 1mL of culture medium to overflow the microwell.
- Collect the cell spheroids by pipetting the culture medium.

Notes:

* If spheroids do not easily release out from the microwells, dislodge the TASCL from the culture insert by holding the rim of the TASCL microplate with a pair of forceps. Use medium or buffer and try pipetting again.

* If the spheroids are stuck to the membrane of the culture insert, separate TASCL from the culture insert and use a cell-scraper. Adhesion to the membrane occurs more frequent when cultured cells produce a lot of matrix, such as chondrocytes, or cells are cultured for a longer period.

* Collected spheroids may stick together by centrifugation. Immediately resuspend the spheroids to avoid coagulation.

If you have any questions, please contact IWAI NORTH AMERICA (info@iwai-chem.com).