TASCL Manual

January 2023 version

The following is a standard protocol, adjust for your application.

[About the purpose of using TASCL]
TASCL is currently available for research purposes. In principle, administration of TASCL-cultured cell aggregates to humans is not permitted, except for some clinical studies. If you are considering clinical research on humans, please contact us.

Materials to prepare in the clean bench or the safety cabinet

- Packed TASCL
- Pipette
- Remove air bubbles with a water flow such as a 100 µl to 1000 µl pipette or syringe for removing air bubbles Equipment for (If removing air bubbles with an incubator or centrifuge, these are not required, see step 2)
- Culture medium
- Sterilized water
- Collection container for excess sterilized water and medium
- When seeding cells, use a cell suspension(see step 3)
- When collecting cell clumps, a container to put the collected cell clumps in

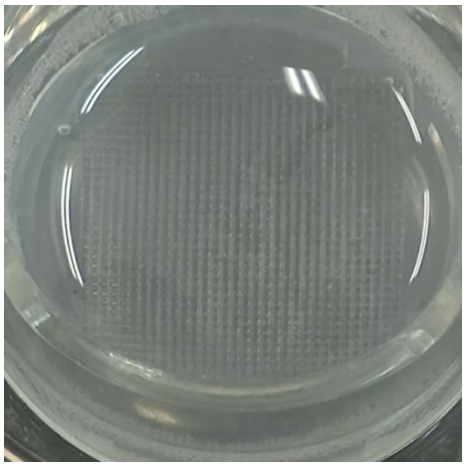
1. Check for air bubbles

With a pipette, add about 2 ml of sterilized water to the entire microwell of TASCL. Check how many bubbles are formed inside the microwell.

If bubbles are found in 5% or more of the entire microwell, remove the bubbles in the next "2. Remove bubbles". If there are few bubbles, proceed to "3. Cell seeding".

Bubbles on microwells

No bubbles on microwells



2. Remove bubbles in microwells

Remove air bubbles by either method A, B, or C.

A. Remove air bubbles in the incubator

Place the TASCL with sterilized water (or medium) in the incubator at 37°C for about 30 minutes in step 1. After 30 minutes, remove the TASCL from the incubator.

Check the presence of air bubbles in the microwells, and if the air bubbles have almost disappeared (when the air bubbles are less than 5% of the entire microwell), proceed to "3. Adjusting the cell suspension".

If many bubbles remain in the microwells, try method B or C.

B. Remove air bubbles with pipette water flow

Add sterilized water onto the TASCL (up to about half the height of the culture insert), suck the sterilized water with a pipette, blow it strongly on the air bubbles, and remove the air bubbles with a water flow. (You can use disposable syringes instead of pipettes.)

When the bubbles in the microwells have almost disappeared (when the bubbles are less than 5% of the entire microwells), proceed to "3. Adjusting the cell suspension".

If there are still many bubbles, please try method C.

C. Centrifuge to remove air bubbles

In step 1, the TASCL with the sterilized water (or culture medium) still in it is put on the lid and centrifuged. Air bubbles can be removed by centrifuging at 2000 RPM for about 5 minutes.

When most of the bubbles in the microwells have disappeared (when the bubbles are less than 5% of the entire microwells), proceed to “3. Adjusting the cell suspension”.

3. Prepare the cell suspension

Prepare the cell suspension as follows.

TASCL 600 Wells:

Suspend 621 cells per microwell in 0.5 ml medium.

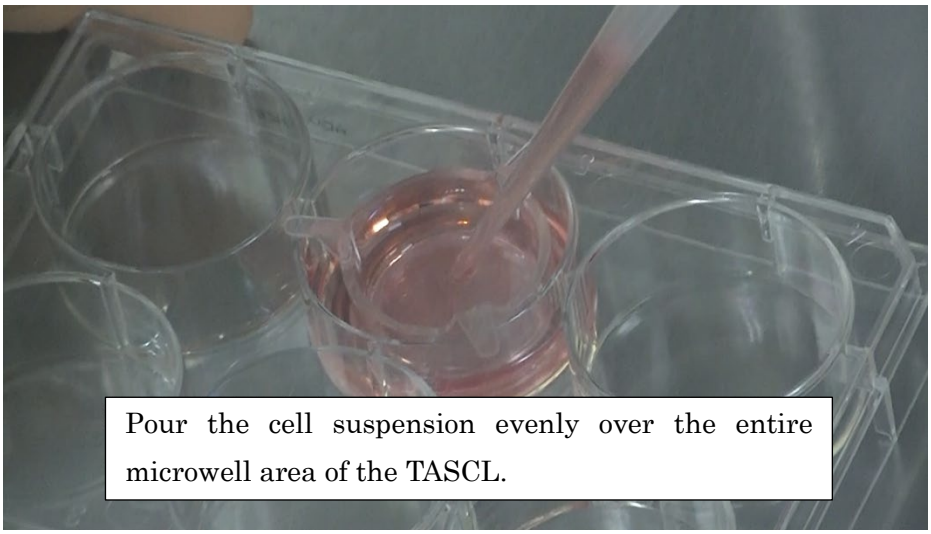
TASCL 1000 wells:

Suspend 1020 cells per microwell in 0.5ml medium.

4. Cell the cell suspension

Aspirate the sterile water remaining in the microwells and on the 6-well plate with a pipette, rinse the microwells and 6-well plate with medium, and aspirate the medium. Inject about 2 ml of medium into the microwell.

Drop the cell suspension prepared in step 3 over the entire surface of the TASCL mesh and wait until the cells settle to the bottom of the mesh. Cell sedimentation depends on the specific gravity of the cells and the medium, so it is recommended to check with an inverted microscope each time. In most cases, cells settle within 30 minutes.



Pour the cell suspension evenly over the entire microwell area of the TASCL.

5. Adding medium

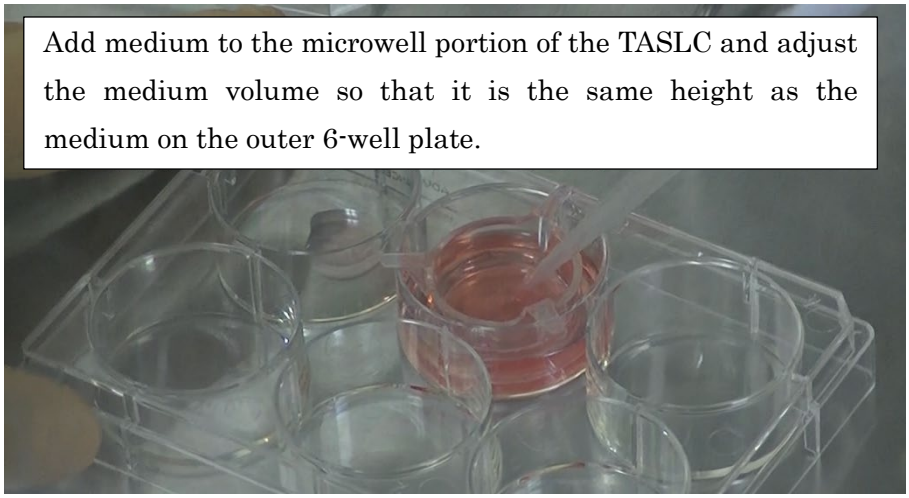
Remove the culture insert together with TASCL from the 6-well plate, or add 3.0 ml of medium to the outside of the culture insert (on the 6-well plate) through the gap in the culture insert.

To prevent the surface of the TASCL from drying out due to medium evaporation,

add a small amount of medium to the inside of the TASCL and the outside of the culture insert so that the liquid level inside the TASCL and the outside of the culture insert (on a 6-well plate) are the same. Add one by one and adjust.

In the future, even if the medium evaporates and decreases, the medium will be added in the same way.

*Make sure that the liquid level of the medium on the outside is at least the same height as the liquid level of the medium on the TASCL. If the liquid level is low, the medium on the TASCL may flow out due to the siphon effect during long-term culture.
*If it is necessary to warm the medium, put it in the incubator for about 10 minutes beforehand.



6. Medium change

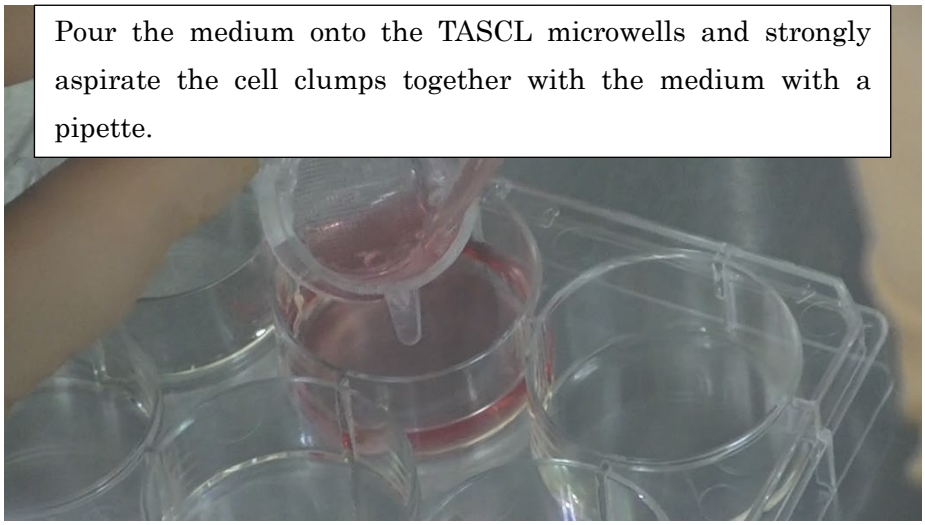
Use a pipette to aspirate the medium in the 6-well plate.
(You can also aspirate through the gap between the outside of the culture insert and the 6-well plate without removing the culture insert.)
Add a new medium for a height of about 3 mm.
Set the culture insert.
Add medium to the mesh portion of the TASCL and adjust to the same level as the medium on the outer 6-well plate.
Medium exchange is now complete.

7. Recovery of spheroids

Aspirate and dispense the medium several times with a pipette, remove the spheroids from the TASCL, and aspirate the spheroids together with the medium.

Rinse TASCL by dispensing fresh medium into TASCL. Peel off the spheroids and pipette again to aspirate the spheroids with the medium.

If spheroids remain in the TASCL, aspirate the medium vigorously.
Repeat the above steps several times, as spheroids tend to remain in the square corners of the microwell.



Pour the medium onto the TASCL microwells and strongly aspirate the cell clumps together with the medium with a pipette.

If it is difficult to aspirate the spheroids, pull the disc-shaped silicone part of the TASCL with tweezers and remove it from the culture insert.

Then, the whole TASCL body can be rinsed with the medium, and the spheroids peeled off from the TASCL can be collected together with the medium.

[Contact: TASCL manufacturer and distributor]

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