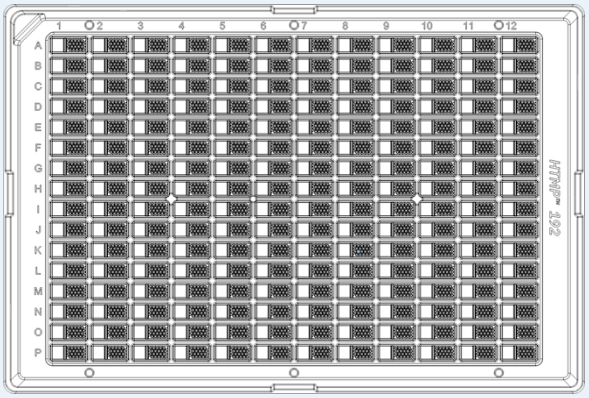


HTMP™ BIOTECHNOLOGY

HTMP™ 192

High-throughput microwell platform for physiologically relevant 3D cell culture.

Product Description

The HTMP™ 192 platform is designed to generate and maintain cellular aggregates, including spheroids, microtissues, assembloids, and, in some contexts, organoids (cellular bodies that exhibit lumen formation), for extended culture and downstream experimentation. Each rectangular well spans two positions of a standard 384-well plate while maintaining a 384-well pitch pattern. For plate readers, multichannel pipettes, and automated imaging systems, data acquisition should therefore be targeted to A2, B2, C2, and every other well thereafter. Each well contains a pipette access area and a microwell field separated by a partition wall. This partition wall enables media exchange through the pipette access area while minimizing disturbance to, or loss of, cellular material within the microwell field. The microwell field contains 20 semi-conical microwells that support uniform aggregate formation. Aggregate size can be tuned based on the number of cells seeded per microwell.

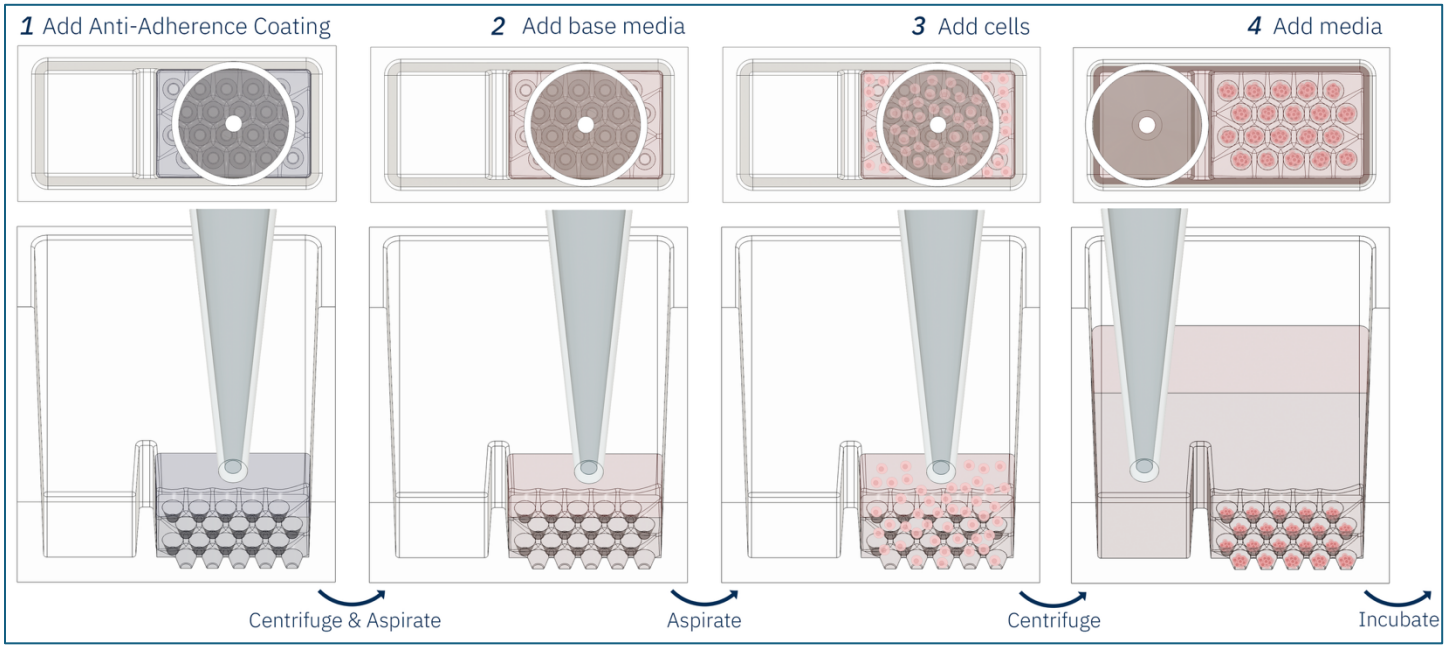
Storage and Stability

Store HTMP™ 192 plates at room temperature (15-25 °C) away from direct sunlight. Plates are stable for 5 years from the date of manufacture shown on the product label.

Basic Product Information

Name	Catalog No.	Size	Description
HTMP™ 192	#0001	1 plate	192 wells; 20 microwells per well; microwells are 600 µm (top diameter) x 350 µm (bottom diameter) x 400 µm deep.

Protocol Schematic



Materials Required but not Included

- HTMP™ Cellular Anti-Adherence Coating (5 mL) - 15 µl per well
- Trypsin / EDTA
- Trypan Blue
- Cell Counting Slides and hemocytometer or cell counter
- Serological Pipettes
- Conical 5 mL & 50 mL Tubes
- Optional: WST-8 Solution for viability assessment (APEx Bio, K1018)

Protocol

Preparation

1. Remove cells from the incubator and place them in a biosafety cabinet.
2. Aspirate the culture medium.
    - **Optional**: wash 1x with pbs.
3. Add 0.25% - 0.5% Trypsin / EDTA to the cells.
4. Return the cells to the incubator for approximately 5 minutes or until the cells have detached.

5. After detachment, pipette up and down vigorously with a p1000 to dissociate clumps and obtain a single-cell suspension.
6. Transfer the cells to a 5 mL or 50 mL conical tube with an equal volume of cell culture media to neutralize the trypsin.
7. Centrifuge at 500 x g for 5 minutes.
8. Aspirate the supernatant and resuspend the cell pellet in 1ml of culture media.
9. Perform a cell count using Trypan Blue and a hemocytometer to determine the viable cell concentration.
10. Use Table 1 to calculate the number of cells required per well.
11. Prepare a master mix of cells at the appropriate concentration
12. Cells are seeded in 20 µl per well
    - **Example**: To seed 30 wells at 300 cells per microwell, you need 6,000 cells per well (20 microwells x 300 cells), or 180,000 total cells in 600 µL of medium.
    - **Tip:** Prepare enough cell suspension for 5-10 extra wells to account for pipetting loss.

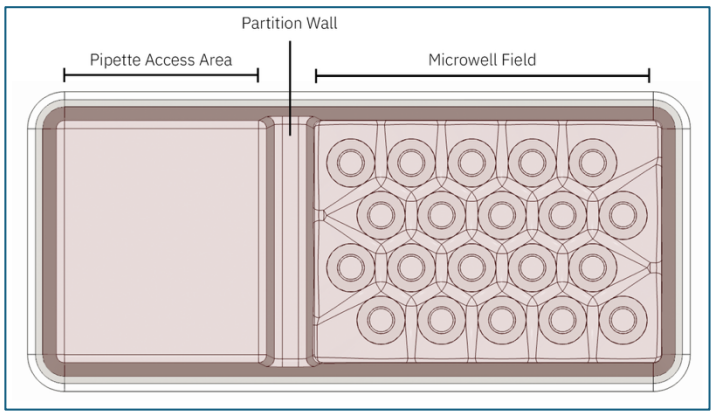
Table 1. Number of cells needed per well in the HTMP™ 192.

Desired cells per microwell	Total cells per well
100	2,000
200	4,000
300	6,000
400	8,000
500	10,000

Seeding

1. Unbox the HTMP™ 192 and place inside the biosafety cabinet.
2. Add 15 µL of HTMP™ Cellular Anti-Adherence Coating to the microwell field of each well to be used.

Single-well schematic



3. Centrifuge the plate at 1,000 x g for 5 minutes to remove bubbles from the microwells.
4. Inspect the plate under a microscope to confirm that bubbles have been removed from all microwells.
5. Aspirate the anti-adherence coating from the microwell field.
    - **TIP**: When aspirating the anti-adherence coating, do not sweep the entire field. Aspirate from a single corner or from the center of the well.
6. Wash once by adding 20 µL of cell culture medium to the microwell field and aspirating from the same location as in step 5.
7. Add 20 µl of the resuspended cell master mix to the microwell field.
8. After loading all wells, centrifuge the plate at 150 x g for 5 minutes to seed cells uniformly into each microwell.
9. Add 130 – 180 µL of cell culture media to the pipette access area.
    - **TIP**: dispense the cell culture media at a rate of < 50 µL/s.
10. Place the HTMP™ 192 plate in the incubator.

Media Exchange

1. Perform the first media change 24 hours after seeding. After that, replace media every 48 hours or as appropriate for your cell type and assay.
2. Remove the plate from the incubator and place in a biosafety cabinet.
3. Aspirate media through the pipette access area.
    - **IMPORTANT**: Do not aspirate media over the microwell field, as this may result in cell or microtissue loss or displacement.
4. Add 130 – 180 µL of fresh culture media to the pipette access area at a rate < 75 µL/ s.
5. Return the HTMP™ 192 back in the incubator.

Viability Assessment via WST-8 (Optional)

1. For toxicity or viability screening, perform WST-8 analysis 72 hours or later to allow for adequate microtissue maturation.
2. Prepare a viability solution containing 10% WST-8 reagent in cell culture medium.
3. Remove the plate from the incubator and place in a biosafety cabinet.
4. Aspirate media through the pipette access area.
    - **IMPORTANT**: Do not aspirate media over the microwell field, as this may result in cell or microtissue loss or displacement.
5. Add 130 – 180 µL of the WST-8 solution to each well.
6. Return the plate to the incubator for 2 – 4 hours.
    a. **Optional TIP**: During incubation, gentle mixing above the microwell field may help distribute the WST-8 reagent more evenly. Avoid vigorous pipetting if aggregate displacement is a concern for your cell type or culture condition.
7. Transfer 100 µL of supernatant from each well into a clear-bottom 96-well plate.
8. Measure the absorbance at 450 nm using a microplate reader.