AggreWell™ HT

Microwell culture plates for high-throughput, easy, and reproducible production of embryoid bodies and spheroids

Catalog #200-0563	1 Unit
Catalog #200-0570	5 Units

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Product Description

AggreWell™ HT plates are used to generate cell aggregates such as embryoid bodies (EBs) and spheroids. Each well of the 96-well AggreWell™ HT plate contains a standardized array of 32 microwells. The size of the EBs and spheroids formed is highly uniform and can be easily controlled by adjusting the input cell density. AggreWell™ HT plates can support the input cell number ranges of both the AggreWell™ 400 and AggreWell™800 formats. AggreWell™ HT plates can be used to perform high-throughput experiments of applications previously developed to tested in all other AggreWell™ formats.

NOTE: For all cell types, **Anti-Adherence Rinsing Solution (Catalog #07010) is required** during plate preparation steps to ensure optimal performance. Anti-Adherence Rinsing Solution prevents cell adhesion and promotes efficient formation of EBs and spheroids.

Storage and Stability

Store AggreWell™ HT plates at room temperature (15 - 25°C) away from direct sunlight. Stable for 5 years from date of manufacture (MFG) on label.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
37 µm Reversible Strainer	27215 (Small) OR 27250 (Large)
Anti-Adherence Rinsing Solution	07010
Corning® Filtered Pipette Tips, 200 µL	38032
Falcon® Conical Tubes	38009 (15 mL) OR 38010 (50 mL)
Falcon® Serological Pipettes	38002 (2 mL) OR 38003 (5 mL)
Wide-bore disposable pipette tips, 200 µL	e.g. Fisher Scientific 14-222-730
Trypan Blue	07050

Directions for Use

NOTE: Do not expose AggreWell™ HT plates to organic solvents, including ethanol or isopropanol.

A. MEDIUM FOR EBs OR SPHEROIDS

Select an appropriate medium as follows:

- For generation of **EBs**:

  It is essential to start with a high-quality population of undifferentiated embryonic stem (ES) or induced pluripotent stem (iPS) cells. Use an EB formation medium (e.g. AggreWell™ EB Formation Medium, Catalog #05893) supplemented with 10 µM Y-27632 (Dihydrochloride; Catalog #72302).

- For generation of **spheroids** from other cell types (including cancer spheroids):

  Select an appropriate culture medium for the desired downstream application. If serum-free medium is desired, MammoCult™ Medium (Catalog #05620) may be used.

When warming medium, warm to room temperature (15 - 25°C) or 37°C as directed in the Product Information Sheet for the applicable medium.

Please read the entire protocol before proceeding.

Use sterile technique when performing the following protocols:

B. Preparation of AggreWell™ HT Plates

C. Generation of EBs or Spheroids

D. Changing Medium in AggreWell™ HT Plates

E. Harvesting EBs/Spheroids from AggreWell™ HT Plates

B. PREPARATION OF AGGREWELL™ HT PLATES

NOTE: For all cell types, Anti-Adherence Rinsing Solution is required during plate preparation steps to ensure optimal performance. Anti-Adherence Rinsing Solution prevents cell adhesion and promotes efficient formation of EBs and spheroids.

1. Warm an appropriate medium (see section A).
2. Open AggreWell™ HT plates in a biosafety cabinet.

   NOTE: Do not expose AggreWell™ HT plates to organic solvents, including ethanol or isopropanol.
3. Pre-treat wells with Anti-Adherence Rinsing Solution as follows:
   a. Add 75 µL of Anti-Adherence Rinsing Solution to each well to be used.
   b. Centrifuge plate at 1300 x g for 5 minutes in a swinging bucket rotor fitted with plate holders.

   NOTE: Plates must be well balanced. Prepare a balance plate using a standard plate filled with water to match the weight and position of the AggreWell™ HT plate.
   c. Observe the plate under a microscope to ensure that bubbles have been removed from microwells. If bubbles remain trapped in any microwells, centrifuge at 1300 x g for an additional 5 minutes.
   d. Aspirate Anti-Adherence Rinsing Solution from the wells.
4. Rinse each well with 200 µL of warm medium.
5. Aspirate the medium and add 200 µL of warm medium to each well to be used.

C. GENERATION OF EBs OR SPHEROIDS

1. Prepare a single-cell suspension in an appropriate medium (see section A).
2. Perform a cell count to determine the viable cell concentration by using Trypan Blue and a hemocytometer (e.g. Catalog #100-1181) or an equivalent method.
3. Refer to Table 1 to determine the number of cells required per well to achieve the desired number of cells per microwell. Alternatively, calculate the desired input cell volume, as follows:

   96-well plate: *Required number of cells per well = Desired number of cells per microwell x 32 microwells per well*

Table 1. Required Number of Cells per Well for AggreWell™ HT Plates

AggreWell™ HT	
DESIRED NUMBER OF CELLS PER **MICROWELL***	REQUIRED NUMBER OF CELLS PER **WELL**
50	1600
100	3200
200	6400
500	16,000
1000	32,000
2000	64,000
3000	96,000
4000	128,000
5000	160,000
10,000	320,000

*The recommended range is 50 - 10,000 cells per microwell.

NOTE: For most cell types forming spheroids, the number of cells per microwell will equal the number of cells per spheroid (i.e. 100% incorporation); for some cell lines or cell types, incorporation may be less than 100%. For EB protocols, not all ES or iPS cells will be incorporated into the aggregate.

4. Adjust the concentration of the single-cell suspension and add a sufficient volume to each well to achieve the desired number of cells per well (Table 1).

   NOTE: Avoid performing multiple dispensing steps from a single aspiration of the cell suspension as this may reduce the accuracy of seeding numbers in each well.
5. Add warm medium to each well to achieve a final volume of 200 µL/well.
6. Prepare a centrifuge balance plate using a standard plate filled with water to match the weight and position of the AggreWell™ HT plate.
7. Pipette cells up and down gently several times to ensure even distribution of cells throughout the well. Be careful not to introduce bubbles into the microwells.
8. Immediately centrifuge the AggreWell™ HT plate at 100 x g for 3 minutes to capture cells in the microwells, using the balance plate prepared in step 6.
9. Observe plate under a microscope to verify that cells are evenly distributed among the microwells.
10. Incubate the plate at 37°C with 5% CO_2 and 95% humidity for 24 hours. Observe the cells under a microscope.

    NOTE: Although many cell lines form EBs/spheroids within 24 hours, some may require a longer incubation time (i.e. up to 48 hours) for optimal EB/spheroid formation.

D. CHANGING MEDIUM IN AGGREWELL™ HT PLATES

Some applications of EBs or spheroids may require continuous culture in AggreWell™ HT plates, including medium changes. For best results, use 200 µL pipette tips for medium changes.

Perform medium changes as described below:

1. Warm complete medium.
2. Perform a 50 - 75% medium change as follows:
   a. Slowly remove 100 - 150 µL of medium from each well.
   b. Replace with 100 - 150 µL of fresh complete medium by slowly pipetting near the top of the remaining medium level. Slowly dispensing the medium helps to prevent displacement of EBs/spheroids from the microwells.

For culturing cells in AggreWell™ HT plates, refer to the appropriate culture protocol for further instructions. For example, for neural induction of human pluripotent stem cells using STEMdiff™ Neural Induction Medium (Catalog #05835), refer to the Technical Manual: Generation and Culture of Neural Progenitor Cells using the STEMdiff™ Neural System, available at www.stemcell.com or contact us to request a copy.

For harvesting EBs/spheroids from AggreWell™ HT plates, proceed to section E.

E. HARVESTING EBs/SPHEROIDS FROM AGGREWELL™ HT PLATES

1. Warm an appropriate medium.
2. Remove approximately half of the culture medium from the well.
3. Using a 200 µL wide bore pipette tip, dispense the medium firmly back onto the surface of the plate to dislodge the EBs/spheroids from the microwells. Do not triturate.
4. Select the appropriate strainer and conical tube for separation of EBs/spheroids from single cells:
   - For harvesting from a single well, use 37 µm Reversible Strainer, Small and a 15 mL conical tube
   - For harvesting and pooling from multiple wells, use 37 µm Reversible Strainer, Large and a 50 mL conical tube
5. Place the strainer on top of the tube with the arrow pointed upward.
6. Using a 200 µL wide bore pipette tip, gently aspirate the dislodged EBs/spheroids (from step 3). Pass the EB/spheroid suspension through the strainer.

   NOTE: The aggregates will remain on the filter; any unincorporated single cells will flow through.
7. Dispense 200 µL of warm medium across the entire surface of the well to dislodge any remaining EBs/spheroids. Collect wash and pass over the strainer used in step 6. Repeat this wash step 3 times.
8. Invert the strainer, and place over a new conical tube of the same size. Collect the EBs/spheroids by washing with 1 - 5 mL of complete medium per well harvested as appropriate.
9. Observe the AggreWell™ HT plate under a microscope to ensure that all EBs/spheroids have been removed from the wells. Repeat wash if necessary (i.e. steps 7 - 8).
10. OPTIONAL: Count the EBs/spheroids to determine actual yield, as described below.

    Using a serological pipette:
    a. Pipette the EB/spheroid suspension up and down 2 - 3 times to ensure even distribution.
    b. Pipette 50 µL of the EB/spheroid suspension into a flat-bottom 96-well plate. Count at 20 - 100X magnification.
    c. Calculate EB or spheroid yield as follows:

$$Total\ number\ of\ EBs\ or\ spheroids = \frac{EB\ or\ spheroid\ count\ (in\ 50\ \mu L)}{50\ \mu L}\ x\ Volume\ of\ EB\ or\ spheroid\ suspension\ (\mu L)$$

    NOTE: The expected yield is 32 EBs/spheroids for 1 well.
11. Harvested EBs/spheroids are now ready for downstream analysis and applications, such as suspension culture, directed differentiation (EBs), drug screening and toxicity assays.

F. SCREENING EBs/SPHEROIDS IN AGGREWELL™ HT PLATES

Screening can be performed in a variety of ways. For example:

- To study the effect of treatments on EB or spheroid formation, compounds can be added to the medium used to create the single-cell suspension in section C.
- To test the effects of compounds on pre-formed EBs or spheroids, including EBs differentiated to a specific lineage or target cell type in AggreWell™ HT plates, it is recommended to dilute compounds in an appropriate medium at 2X final concentration before adding to the culture during a 50% medium exhange.