AggreWell™800

Microwell culture plates for easy and reproducible production of embryoid bodies and spheroids

Catalog #34811	1 Plate
Catalog #34815	5 Plates
Catalog #34850	1 Kit

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Product Description

AggreWell™ plates are used to generate cell aggregates such as embryoid bodies (EBs) and spheroids. Each well contains a standardized array of microwells, enabling the production of large numbers of EBs and spheroids. The size of the EBs and spheroids is highly uniform and can be easily controlled by adjusting the input cell density.

AggreWell™ plates can be used to generate uniform aggregates of any cell type, which helps to ensure reproducibility of experiments.

NOTE: For all cell types, **AggreWell™ Rinsing Solution (Catalog #07010) is required** during plate preparation steps to ensure optimal performance. AggreWell™ Rinsing Solution prevents cell adhesion and promotes efficient EB and spheroid formation.

Storage and Stability

Store AggreWell™ plates at room temperature (15 - 25°C) away from direct sunlight. Stable for 2 years from date of manufacture on label.

Product Information

PRODUCT NAME	CATALOG #	SIZE	DESCRIPTION
AggreWell™800 24-well Plate	34811	1 plate	24 wells, approximately 300 microwells per well. Microwells are 800 µm in size.
	34815	5 plates	
AggreWell™800 24-well Plate Starter Kit	34850	1 Kit	• 2 x AggreWell™800 24-well Plates (Catalog #34811) • 1 x AggreWell™ Rinsing Solution (100 mL; Catalog #07010)

Materials Required But Not Included

PRODUCT NAME	CATALOG #
AggreWell™ Rinsing Solution*	07010
Trypan Blue	07050
37 µm Reversible Strainer	27215 (Small) OR 27250 (Large)
Conical tubes	Corning 352070 (50 mL) OR Corning 352196 (15 mL)

*Included in AggreWell™800 24-well Plate Starter Kit (Catalog #34850)

Directions for Use

Please read the entire protocol before proceeding.

- For generation of **EBs**:

  Use an appropriate EB formation medium (e.g. AggreWell™ EB Formation Medium, Catalog #05893). It is essential to start with a high-quality population of undifferentiated embryonic stem (ES) or induced pluripotent stem (iPS) cells.
- 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™ (Catalog #05620) may be used.
- When warming medium, warm to room temperature or 37°C as appropriate.

Use sterile techniques when performing the following protocols:

A. Preparation of AggreWell™ Plates
B. Generation of EBs or Spheroids
C. Changing Medium in AggreWell™ Plates
D. Harvesting from AggreWell™ Plates

A. PREPARATION OF AGGREWELL™ PLATES

NOTE: For all cell types, AggreWell™ Rinsing Solution is required during plate preparation steps to ensure optimal performance. AggreWell™ Rinsing Solution prevents cell adhesion and promotes efficient EB and spheroid formation.

1. Warm basal medium and complete medium.
2. Pre-treat wells with AggreWell™ Rinsing Solution as follows:
   a. Add 500 µL of AggreWell™ Rinsing Solution to each well to be used.
   b. Centrifuge plate at 2000 x *g* (or at maximum speed) 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™ plate.
   c. Observe plate under a microscope to ensure that bubbles have been removed from microwells. If bubbles remain trapped in any microwells, centrifuge again at higher speed (or maximum speed) for an additional 5 minutes.
   d. Aspirate AggreWell™ Rinsing Solution from the wells.
3. Rinse each well with 2 mL of warm basal medium. Aspirate medium from the well.
4. Add 1 mL of warm complete medium to each well to be used.

B. GENERATION OF EBS OR SPHEROIDS

1. Prepare a single-cell suspension in desired medium.
2. Perform a cell count using Trypan Blue and a hemocytometer to determine the viable cell concentration.
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 using the following formula:

   Required number of cells per well = Desired number of cells per microwell x 300 microwells per well

Table 1. Required Number of Cells per Well for AggreWell™800 24-well Plates

DESIRED NUMBER OF CELLS PER **MICROWELL***	REQUIRED NUMBER OF CELLS PER **WELL**
3,000	9.0 x 10^5
4,000	1.2 x 10^6
5,000	1.5 x 10^6
10,000	3.0 x 10^6

*The recommended range is 3,000 - 20,000 cells per microwell.

NOTE: For most cell types, 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 EBs, 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 of cell suspension to each well to achieve the desired cell number as per 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 complete medium to each well to achieve a final volume of 2 mL/well.
6. Prepare a centrifuge balance plate using a standard plate filled with water to match the weight and position of the AggreWell™ 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™ 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: Many cell lines form EBs/spheroids within 24 hours, but some may require a longer incubation time (up to 48 hours) for optimal EB/spheroid formation.

C. CHANGING MEDIUM IN AGGREWELL™ PLATES

Some applications of EBs or spheroids may require continuous culture in AggreWell™ plates, including medium changes. For best results, use wide-bore 1000 µL pipette tips or 2 - 5 mL serological pipettes for medium changes.

Perform medium changes as described below:

1. Warm complete medium.
2. Perform a 50 - 75% medium change as follows:
   a. Slowly aspirate 1 - 1.5 mL of medium from each well.
   b. Replace with 1 - 1.5 mL of fresh complete medium by slowly pipetting down the wall of the well.

   NOTE: Dispensing fresh medium slowly down the wall of the well will help to prevent displacement of EBs/spheroids from the microwells.

If culturing cells in AggreWell™ 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 (Document #28782), available on our website at www.stemcell.com or contact us to request a copy.

D. HARVESTING FROM AGGREWELL™ PLATES

1. Warm basal medium and complete medium.
2. Using a 2 mL serological pipette and Pipet-Aid, or a micropipettor with a 1 mL pipette tip:
   a. Aspirate approximately half of the culture medium from the well.
   b. Dispense the medium firmly back onto the surface of the plate to dislodge the EBs/spheroids from the microwells. Do not triturate.

   NOTE: If using a micropipettor, ensure that the spheroids are not too large for the tip, or they may break apart during collection. For spheroids of greater than 3,000 cells, use large-bore tips (e.g. Rainin Catalog #HR-1000 WS) or aseptically cut the end of a standard 1 mL pipette tip to increase the bore size.
3. 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 from multiple wells, use 37 µm Reversible Strainer, Large and a 50 mL conical tube
4. Place strainer on top of the tube with the arrow pointed upward.
5. Gently aspirate the dislodged EBs/spheroids from step 2. Pass the EB/spheroid suspension through the strainer.

   NOTE: The aggregates will remain on the filter; any unincorporated single cells will flow through.
6. Pipette 1 mL of warm basal medium across the entire surface of the well to dislodge any remaining EBs/spheroids. Collect wash and pass over the strainer used in step 5. Repeat this wash step 3 times.
7. Invert the strainer, and place over a new conical tube of the same size. Collect the EBs/spheroids by washing with 2 - 5 mL of complete medium per well harvested.
8. Observe the AggreWell™ plate under a microscope to ensure that all EBs/spheroids have been removed from the wells. Repeat wash if necessary (steps 6 - 7).
9. OPTIONAL: Count the EBs/spheroids to determine actual yield. The expected yield is approximately 300 EBs/spheroids for one well of an AggreWell™800 plate.
   a. Pipette the EB/spheroid suspension up and down 2 - 3 times to ensure even distribution (see step 2 Note).
   b. Pipette 50 µL of the EB/spheroid suspension into a flat-bottom 96-well plate. Count at 20X - 100X magnification.
   c. Calculate EB or spheroid yield as follows:

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

10. Harvested EBs/spheroids are now ready for downstream applications such as suspension culture, directed differentiation (EBs), drug screening and toxicity assays, and analysis.