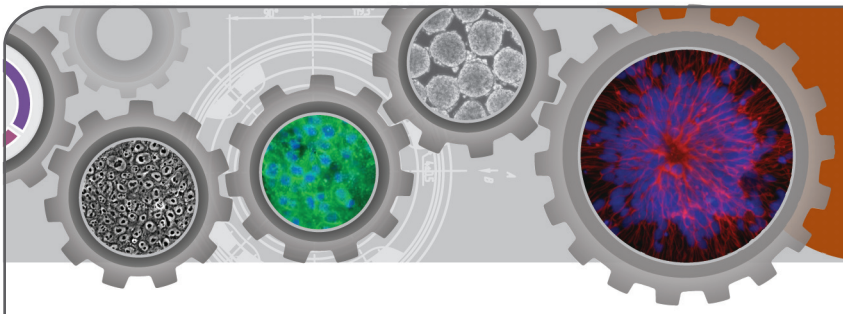


AggreWell™

Reproducible, Uniform Embryoid Bodies

Reproducible Production of Uniformly-Sized Embryoid Bodies

Many pluripotent stem cell differentiation protocols begin with the formation of 3-dimensional aggregates of cells called embryoid bodies (EBs). Conventional EB formation methods[1,2] result in EBs that are heterogeneous in size and shape, leading to inefficient and uncontrolled differentiation[3].

Aggrewell™ plates solve this issue by aggregating pluripotent stem cells into EBs of defined size using microwells.

Embryoid Bodies generated using AggreWell™ Plates are:

- Uniform in size and shape
- Reproducible
- Size-controlled

AggreWell™400 and AggreWell™400Ex plates have microwells 400 µm in diameter, and AggreWell™800 plates have microwells 800 µm in diameter (Figure 1). EB formation is accomplished by adding a single cell suspension to the plate, centrifuging to distribute the cells evenly among the microwells, and then culturing for a minimum of 24 hours to allow aggregation of the cells within each microwell. The resulting EBs are highly uniform in size (Figure 2) and can be efficiently differentiated into a variety of cell types (Figure 3)[4]. AggreWell™ plates bring an easy and standardized approach to the production of EBs, making differentiation experiments more reproducible. EBs and other cell aggregates[5] generated using AggreWell™ plates are consistent in size and shape, and uniform within and between experiments.

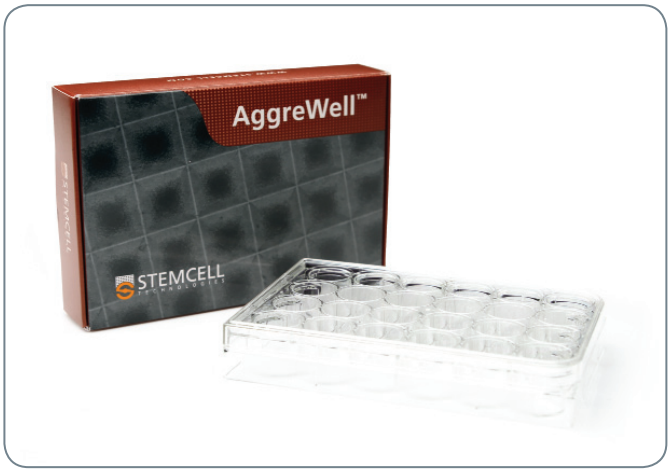
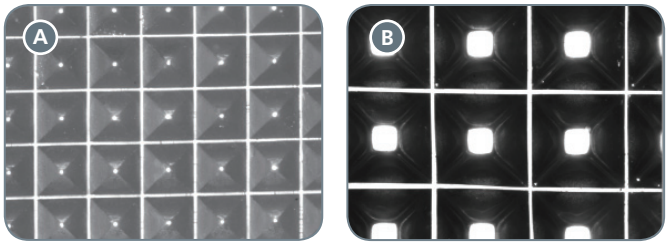


FIGURE 1. AggreWell™ contains microwells to make uniform cell aggregates.



A. AggreWell™400 and AggreWell™400Ex plates contain microwells 400 µm in diameter. Photo taken at 40x magnification.
B. AggreWell™800 plates contain microwells 800 µm in diameter. Photo taken at 40x magnification.

VIDEO

AggreWell™ Introduction

www.stemcell.com/AggreWellVideo

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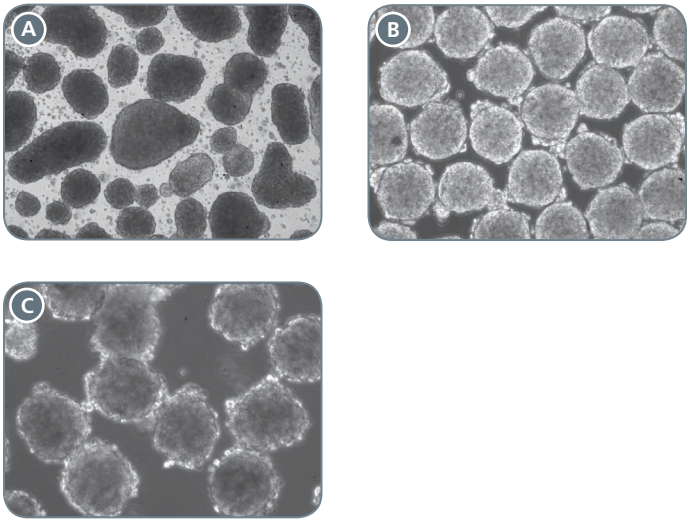
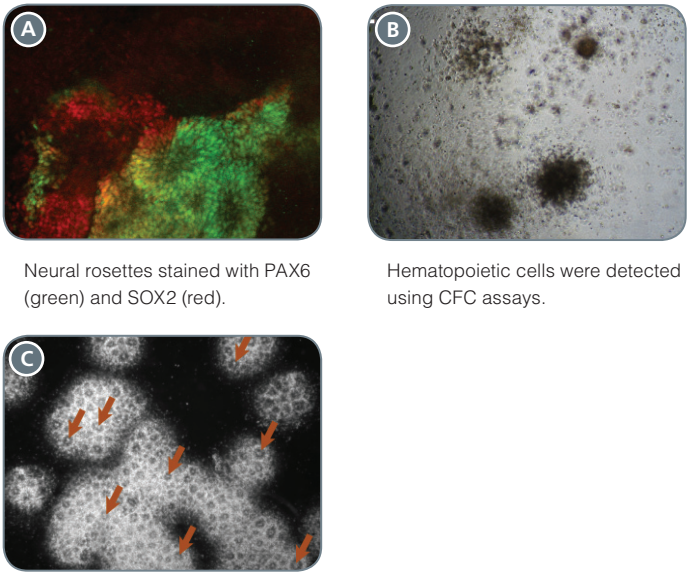
FIGURE 2. Generate uniformly sized embryoid bodies using AggreWell™.



A. Human EBs formed using conventional methods are heterogeneous in size and shape resulting in inefficient differentiation.
B. Human EBs formed using AggreWell™ plates over 24 hours are uniform in size and consistently spherical in shape. Shown are EBs generated with 2,000 cells using AggreWell™400.
C. Mouse EBs (not grown on feeders) formed using AggreWell™400 plates for 72 hours. Shown are EBs generated with 2,000 cells using AggreWell™400. All photos taken at 100x magnification.

FIGURE 3. EBs generated using AggreWell™400 plates are able to differentiate into multiple cell types.



Neural rosettes stained with PAX6 (green) and SOX2 (red).

Hematopoietic cells were detected using CFC assays.

Differentiated cells were generated by culturing AggreWell™400 - generated EBs in serum-containing suspension culture for A. 4 days (neural) or B. 14 days (hematopoietic)*. Neural cells were assayed by plating onto gelatin-coated dishes, and hematopoietic cells were assayed by plating in MethoCult™ (Catalog #04434). C. 100% Neural Induction can be achieved using AggreWell™800 plates and STEMdiff™ Neural Induction Medium (Catalog #05831). Neural aggregates of 10,000 cells each were formed in an AggreWell™800 plate and STEMdiff™ Neural Induction Medium and cultured for 5 days with daily ¾ volume medium changes. Neural aggregates were then harvested and plated onto PLO/L coated plates. Attached neural aggregates are shown 2 days after attachment with prominent neural rosette structures visible (arrows). Magnification 20x.

*Data reprinted from Ungrin et al., 2008.[5,8] See reference for full culture details.

AggreWell™ Products

PRODUCT	DESCRIPTION	USE	QUANTITY	CATALOG#
AggreWell™400 plate	8 wells, each with approximately 1,200 microwells	For EBs composed of 50 to 3000 cells	1/pack	27845
			5/pack	27945
AggreWell™800 plate	8 wells, each with approximately 300 microwells	For EBs composed of 3,000 to 20,000 cells	1/pack	27865
			5/pack	27965
AggreWell™400Ex plate	6 wells, each with approximately 4,700 microwells	For EBs composed of 50 to 3000 cells	1/pack	27840 NEW!
			5/pack	27940
AggreWell™ Medium	Defined, serum-free medium for generation and culture of EBs using AggreWell™ plates		100 mL	05893
AggreWell™ Rinsing Solution†	Rinsing solution for AggreWell™ plates to reduce surface tension. Required for use with AggreWell™400Ex plates.		100 mL	07010 NEW!
37 µm Reversible Strainers, Small	37 µm nylon mesh filter, fits standard 14 mL round bottom tubes and 15 mL conical tubes		20/box	27215 NEW!
37 µm Reversible Strainers, Large	37 µm nylon mesh filter, fits standard 50 mL conical tubes		12/box	27250

† Required only if using AggreWell™400Ex (Catalog #27840/27940). It is not required but can be used with AggreWell™400 (Catalog #27845/27945) or AggreWell™800 (Catalog #27865/27965).

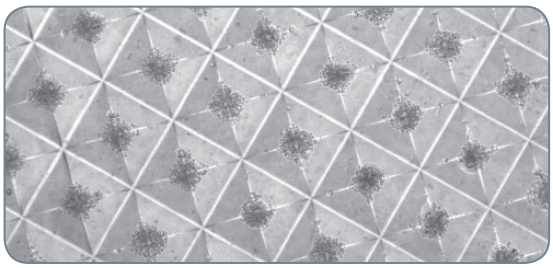
Easily Control the Size of Embryoid Bodies

It has been shown that human as well as mouse EB size directly affects subsequent differentiation trajectories[3,4,6-10]. Since the number of microwells in each well of the AggreWell™ plate is known, it is easy to control the size of the resulting EBs simply by adjusting the number of cells added (Figure 4). Table 1 gives examples of the number of single cells required per well of an AggreWell™400, AggreWell™400Ex and AggreWell™800 plate to generate EBs of varying sizes.

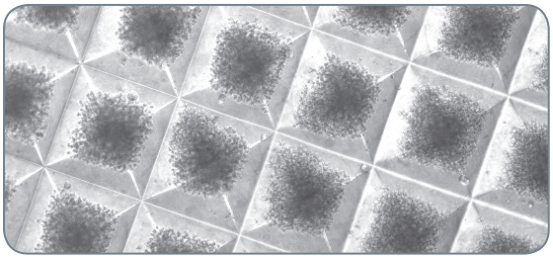
Table 1: Number of PSCs Required to Generate Various Sized EBs Using Aggrewell™400, Aggrewell™400Ex or Aggrewell™800 Plates

DESIRED NUMBER OF CELLS PER EB	REQUIRED NUMBER OF CELLS PER WELL		
	AGGREWELL™400 (Each Well Contains Approximately 1,200 Microwells)	AGGREWELL™400Ex (Each Well Contains Approximately 4,700 Microwells)	AGGREWELL™800 (Each Well Contains Approximately 300 Microwells)
50	6.0 x 10^4 cells	2.3 x 10^5 cells	-
100	1.2 x 10^5 cells	4.7 x 10^5 cells	-
200	2.4 x 10^5 cells	9.4 x 10^5 cells	-
500	6.0 x 10^5 cells	2.3 x 10^6 cells	-
1,000	1.2 x 10^6 cells	4.7 x 10^6 cells	-
2,000	2.4 x 10^6 cells	9.4 x 10^6 cells	-
3,000	3.6 x 10^6 cells	1.4 x 10^7 cells	9.0 x 10^5 cells
4,000	-	-	1.2 x 10^6 cells
5,000	-	-	1.5 x 10^6 cells
10,000	-	-	3.0 x 10^6 cells
15,000	-	-	4.5 x 10^6 cells
20,000	-	-	6.0 x 10^6 cells

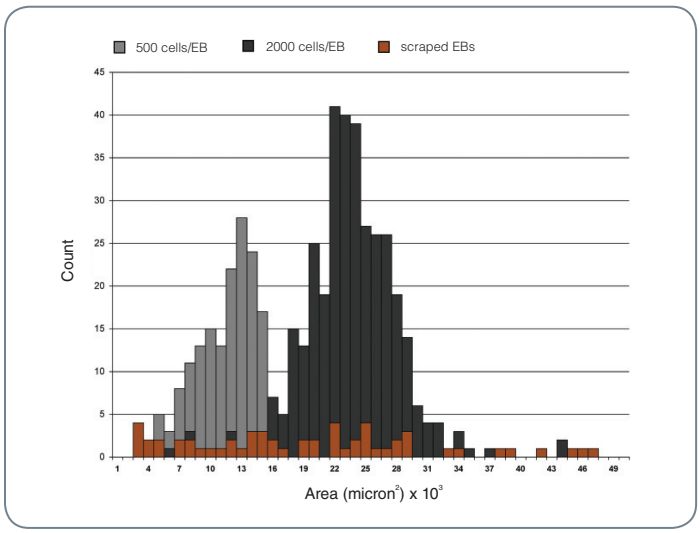
FIGURE 4. The size of EBs can easily be adjusted using AggreWell™ plates. Here, EBs are formed by seeding the wells of an AggreWell™400 plate with varying concentrations of single hESCs so that approximately 500 or 2,000 cells seed each microwell.



500 H9 hESCs seed each microwell.



2000 H9 hESCs seed each microwell.



H9 hESCs were centrifuged into AggreWell™400 plates and cultured for 24 hours prior to EB harvest. EB size is tightly controlled with AggreWell™ (light and dark grey), unlike with scraping protocols (brown) that give a wider distribution.

Optimized AggreWell™ Products

Optimize the generation and isolation of EBs with the use of either AggreWell™ Medium or STEMdiff™ APEL™ Medium and the 37 µm Reversible Cell Strainer. Both AggreWell™ Medium and STEMdiff™ APEL™ Medium support EB formation without serum or added growth factors, and are compatible with mTeSR™1- and TeSR™2-grown cells. After formation in AggreWell™, EBs of greater than 50 cells can be collected in and easily recovered from the 37 µm Reversible Cell Strainer, allowing for easy isolation of EBs from any residual non-incorporated cells.

Rinsing with AggreWell™ Rinsing Solution lowers the surface tension of the AggreWell™ plate, thereby making it easier for air bubbles to be released from the microwells when medium is added and the plate is subsequently centrifuged. The lowered surface tension also makes it easier to remove embryoid bodies (EBs) from the microwells after EB formation. Although the use of AggreWell™ Rinsing Solution is required only when using AggreWell™400Ex plates, it can also be used to rinse AggreWell™400 or AggreWell™800 plates.

Related Products

PRODUCT	CATALOG #
STEMdiff™ APEL™ Medium	05210
STEMdiff™ Neural Induction Medium	05831
STEMdiff™ Neural Rosette Selection Reagent	05832
Y-27632	07171 / 07172
EasySep™ SSEA-4 Positive Selection Kit	18165 / 18145
EasySep™ hESC-Derived CD34 Positive Selection Kit	18167/ 18147
STEMcircles™-LGNSO	05820
Oct 3/4 Antibody, Clone 40	01550 / 01551
SSEA-1 Antibody, Clone MC-480	01552
SSEA-3 Antibody, Clone MC-631	01553
SSEA-4 Antibody, Clone 813-70	01554
TRA-1-60 Antibody, Clone TRA-1-60	01555
TRA-1-81 Antibody, Clone TRA-1-81	01556
TRA-2-49 Antibody, Clone TRA-2-49/6E	01557
TRA-2-54 Antibody, Clone TRA-2-54/2J	01558
mTeSR™1 Defined Maintenance Medium	05850 / 05870 05875 / 05857
mFreSR™ Defined Cryopreservation Medium	05855 / 05854
CryoStor™CS10 Animal Protein-Free Cryopreservation Medium	07930
Dispase (1 mg/mL)	07923

NEW!

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