

REPRODUCIBLY PRODUCE UNIFORM EMBRYOID BODIES

AggreWell™ Plates

Many pluripotent stem cell (PSC) differentiation protocols begin with the formation of 3-dimensional aggregates of cells called embryoid bodies (EBs). EB size directly affects subsequent differentiation trajectories¹⁻⁷, but conventional EB formation methods^{8,9} result in EBs that are heterogeneous in size and shape, leading to inefficient and uncontrolled differentiation¹.

AggreWell™ plates provide an easy and standardized approach to the production of EBs. Each well contains microwells of defined size, making it easy to produce large numbers of highly uniform EBs and ensure reproducibility of differentiation experiments.

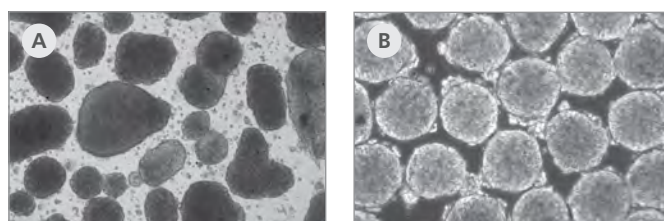


Figure 1. Generate Uniform Embryoid Bodies Using AggreWell™

(A) Human EBs formed using conventional methods are heterogeneous in size and shape resulting in inefficient differentiation. (B) Human EBs formed using AggreWell™ plates are uniform in size and consistently spherical in shape. Shown are EBs generated with 2,000 cells using AggreWell™400.

EBs and other cell aggregates¹⁰⁻¹² generated using AggreWell™ plates are highly uniform in size and shape, and consistent within and between experiments. EB size can be easily modified by adjusting the cell seeding density. EBs formed using AggreWell™ can be efficiently differentiated into a variety of cell types.

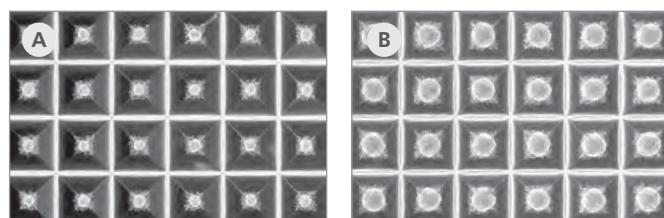


Figure 2. hPSCs Form Embryoid Bodies in AggreWell™ Plates

Starting from a single-cell suspension, hPSCs form uniform EBs after 24 hours in AggreWell™. The size of the EB can be easily modified by adjusting the seeding density. Shown are EBs in AggreWell™400 plates (A) 100 cells per microwell and (B) 1000 cells per microwell.



Why Use AggreWell™?

- EASY TO USE.** Simple EB generation.
- REPRODUCIBLE.** Large numbers of uniform EBs.
- CONTROL OF EB SIZE.** 50 to 20,000 cells per EB.
- CONSISTENCY.** Reduces variability in differentiation protocols.
- HIGH YIELD.** Up to 5,900 EBs per well.

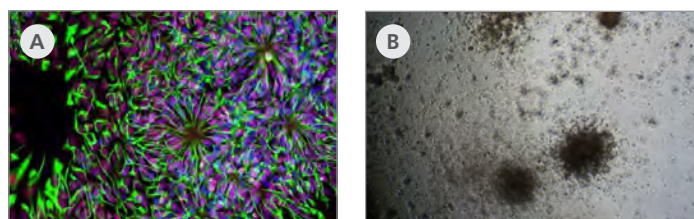
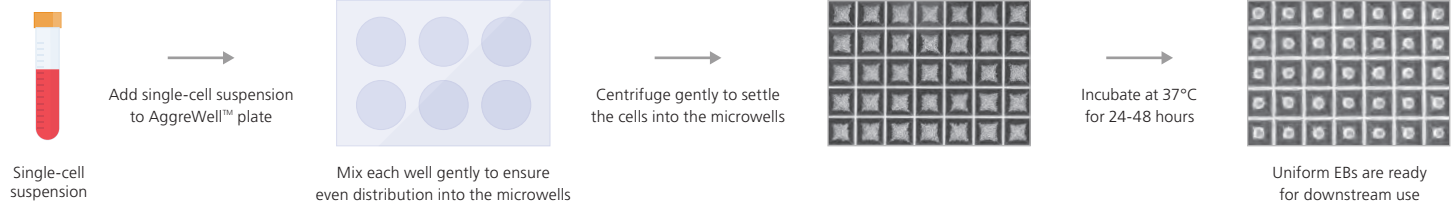


Figure 3. EBs Generated Using AggreWell™ Can Differentiate into Multiple Cell Types

(A) Neural progenitor cells (NPCs) were derived from EBs formed using AggreWell™800 plates and differentiated using STEMdiff™ Neural Induction Medium (Catalog #05835). NPCs express SOX1 (red), Nestin (green) and PAX6 (not shown), but not SOX10 (not shown). Nuclei are counterstained with DAPI. (B)* Hematopoietic progenitor cells were derived from EBs formed using AggreWell™400 plates and differentiated in serum-containing medium in suspension culture. Hematopoietic colony-forming units (CFUs) were detected using MethoCult™ H4435 Enriched (Catalog #04435).

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

Formation of EBs in AggreWell™



AggreWell™ Products

Product	Size of Microwell	Plate Format	Size of EB	Number of EBs	Quantity	Catalog #
AggreWell™400	400 µm	24-well plate	50 - 3,000 cells	~ 1,200 per well	1/pack	34411
					5/pack	34415
		6-well plate		~ 5,900 per well	1/pack	34421
					5/pack	34425
AggreWell™800	800 µm	24-well plate	3,000 - 20,000 cells	~ 300 per well	1/pack	34811
					5/pack	34815
		6-well plate		~ 1,500 per well	1/pack	34821
					5/pack	34825
AggreWell™ EB Formation 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 and ensure optimal EB formation				100 mL	07010
37 µm Reversible Strainers, Small	37 µm nylon mesh filter, fits standard 14 mL round bottom tubes & 15 mL conical tubes				20/box	27215
37 µm Reversible Strainers, Large	37 µm nylon mesh filter, fits standard 50 mL conical tubes				12/box	27250

† Required for optimal performance.

For a complete list of related products for hPSC culture and differentiation, including specialized cell culture and storage media, matrices, antibodies, cytokines, and small molecules, visit www.stemcell.com/hPSCworkflow or contact us at techsupport@stemcell.com.

References

- Bauwens CL et al. (2008) Control of human embryonic stem cell colony and aggregate size heterogeneity influences differentiation trajectories. *Stem Cells* 26: 2300–10.
- Ungrin MD et al. (2008) Reproducible, ultra-high-throughput formation of multicellular organization from single cell suspension-derived human embryonic stem cell aggregates. *PLoS One* 3(2): e1565.
- Bratt-Leal AM et al. (2009) Engineering the embryoid body microenvironment to direct embryonic stem cell differentiation. *Biotechnology Progress* 25: 43–51.
- Hwang YS et al. (2009) Microwell-mediated control of embryoid body size regulates embryonic stem cell fate via differential expression of WNT5a and WNT11. *Proc Natl Acad Sci USA* 106: 16978–83.
- Messana JM et al. (2008) Size of the embryoid body influences chondrogenesis of mouse embryonic stem cells. *J Tissue Eng Regen Med* 2: 499–506.
- Mohr JC et al. (2010) The microwell control of embryoid body size in order to regulate cardiac differentiation of human embryonic stem cells. *Biomaterials* 31:1885–93.
- Ng ES et al. (2005) Forced aggregation of defined numbers of human embryonic stem cells into embryoid bodies fosters robust, reproducible hematopoietic differentiation. *Blood* 106: 1601–03.
- Itskovitz-Eldor J et al. (2000) Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. *Mol Med* 6: 88–95.
- Kurosawa H. (2007) Methods for inducing embryoid body formation: in vitro differentiation system of embryonic stem cells. *J Biosci Bioeng* 103: 389–98.
- Razian G et al. (2013) Production of large numbers of size-controlled tumor spheroids using microwell plates. *J Vis Exp*. 81: e50665.
- Wallace L et al. (2013) Using 3D culture to investigate the role of mechanical signaling in keratinocyte stem cells. *Methods Mol Biol* 989: 153–64.
- Markway BD et al. (2010) Enhanced chondrogenic differentiation of human bone marrow-derived. Mesenchymal stem cells in low oxygen environment micropellet cultures. *Cell Transplantation*. 19: 29–42.

Copyright © 2021 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies and Design, STEMCELL Shield Design, Scientists Helping Scientists, STEMdiff, AggreWell, STEMdiff, and MethoCult are trademarks of STEMCELL Technologies Canada Inc. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.

PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED. FOR ADDITIONAL INFORMATION ON QUALITY AT STEMCELL, REFER TO WWW.STEMCELL.COM/COMPLIANCE.