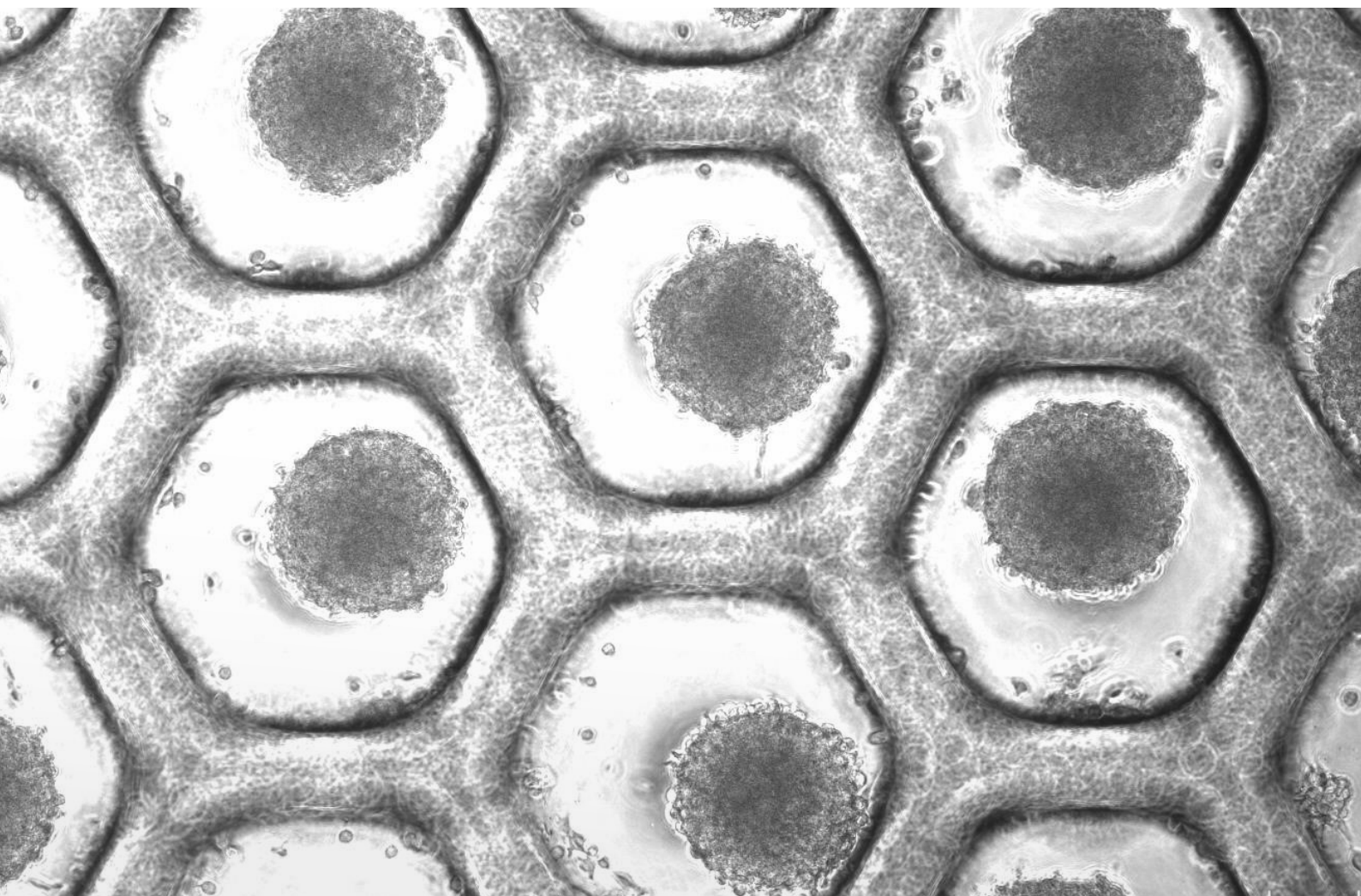


StemFIT 3D®

Protocol & Specification (V2026.07)

Model.

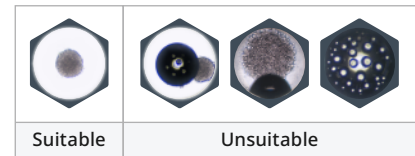
H1613200, H853400, H389600L, H389600H, H449800, H2951000
C100600, C163000, C253000



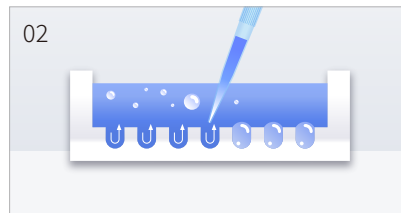
StemFIT 3D® Standard-well protocol

Model. H1613200, H853400, H389600L, H389600H, H449800, H2951000, C100600

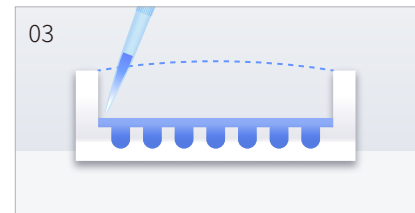
Air Bubble Removal Examples



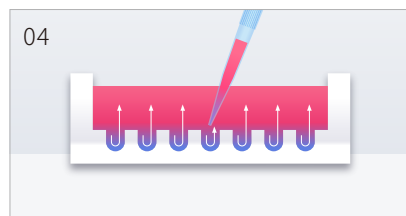
01 Prepare StemFIT 3D and fill it completely with 70% ethanol.



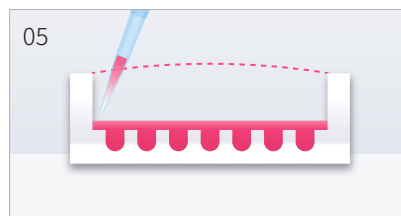
02 Pipette repeatedly toward the microwell bottom to remove air bubbles for smooth cell entry. Verify bubble removal under a microscope.



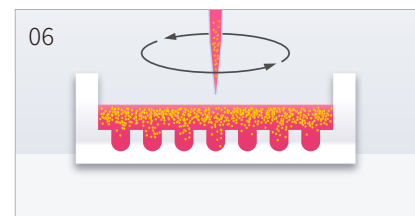
03 Slowly aspirate ethanol from the chamber corner. Leave a small amount of ethanol to prevent microwells from being exposed to air, which may cause re-bubbling.



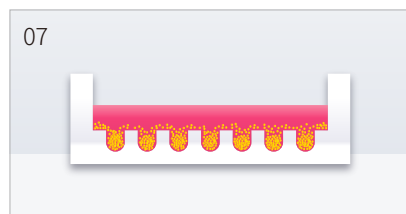
04 Fill with fresh medium through the chamber corner and pipette toward the microwells to dilute the remaining ethanol. Subsequently, prepare a medium containing single cells.



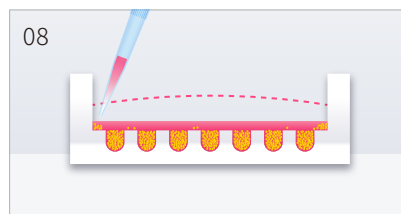
05 Slowly aspirate the diluted medium from the chamber corner. Leave a small amount of medium to keep microwells from being exposed to air.



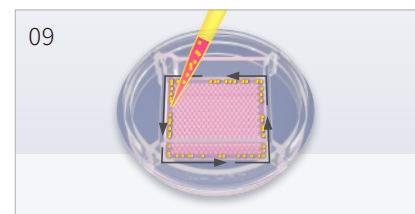
06 For uniform cell distribution, dispense the prepared cell suspension into the center of the chamber all at once.



07 Allow the cells to settle completely at the microwell bottom for about 20 minutes to stabilize. The fewer floating cells there are, the more reproducible spheroids you can obtain.



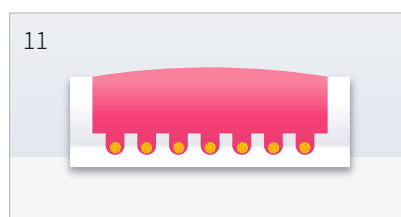
08 Carefully remove the medium through the chamber corner. Aspirate slowly, paying close attention to prevent Medium flow that could wash away the cells inside the wells.



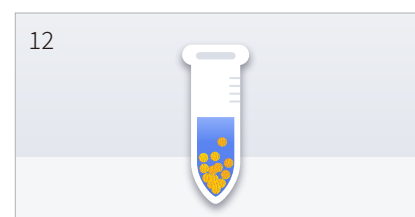
09 After removing the medium, use a pipette tip to aspirate and remove any remaining cells on the edge walls of the chamber along the corners.



10 Fill completely with fresh medium through the chamber corner. Add slowly with the pipette tip placed against the wall to prevent cells from escaping the microwells.



11 Spheroids will form within 5 to 24 hours depending on the cell type. Change the medium through the chamber corner for subsequent culture.

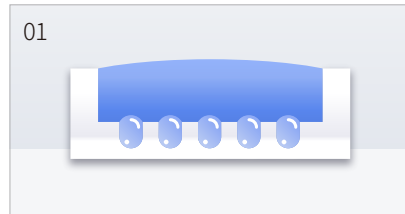
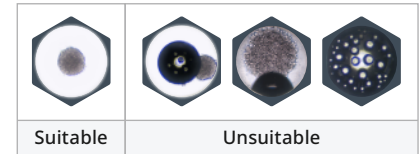


12 Once spheroids are fully formed, pipette gently to detach and harvest them from the microwells. ** If fixation is required for subsequent analysis, fix the structures before detachment.

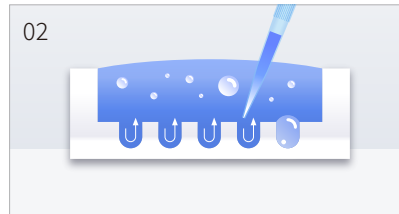
StemFIT 3D® Large-well protocol

Model. C163000, C253000

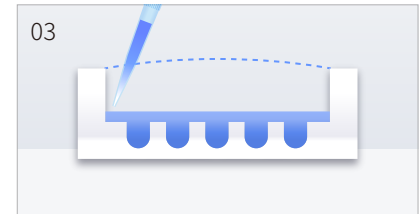
Air Bubble Removal Examples



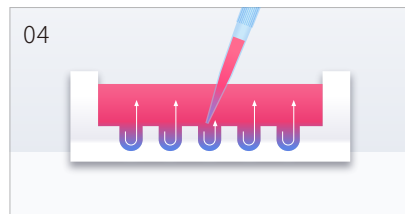
01 Prepare StemFIT 3D and fill it completely with 70% ethanol.



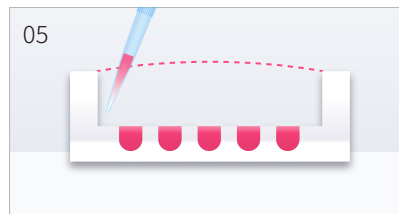
02 Pipette repeatedly toward the microwell bottom to remove air bubbles for smooth cell entry. Verify bubble removal under a microscope.



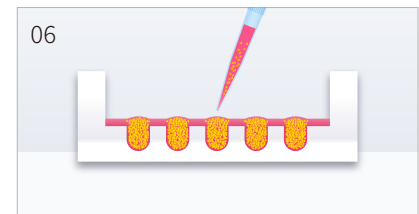
03 Slowly aspirate ethanol from the chamber corner. Leave a small amount of ethanol to prevent microwells from being exposed to air, which may cause re-bubbling.



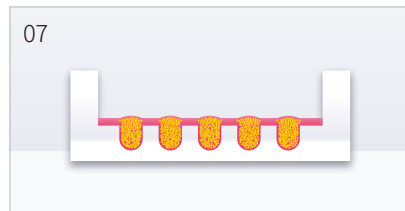
04 Fill with fresh medium through the chamber corner and pipette toward the microwells to dilute the remaining ethanol.



05 Slowly aspirate the diluted medium from the chamber corner. Subsequently, prepare a medium containing single cells.



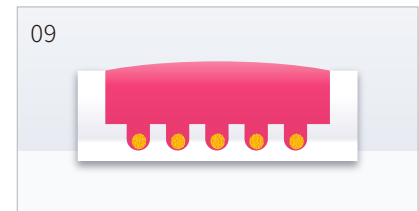
06 Dispense the prepared cell suspension into each microwell.



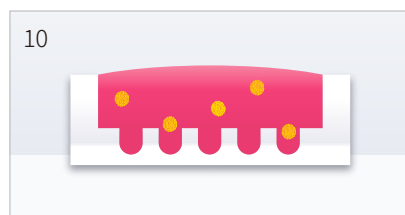
07 Incubate in a CO₂ incubator for 30 minutes.



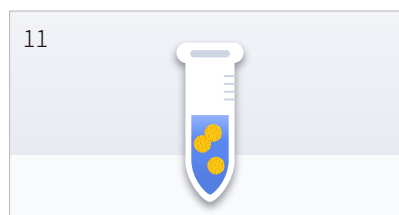
08 Fill completely with fresh medium through the chamber corner. Add slowly with the pipette tip placed against the wall to prevent cells from escaping the microwells.



09 Spheroids will form within 5 to 24 hours depending on the cell type. Change the medium through the chamber corner for subsequent culture.



10 Once spheroids are fully formed, pipette gently to detach and harvest them from the microwells. ** If fixation is required for subsequent analysis, fix the structures before detachment.

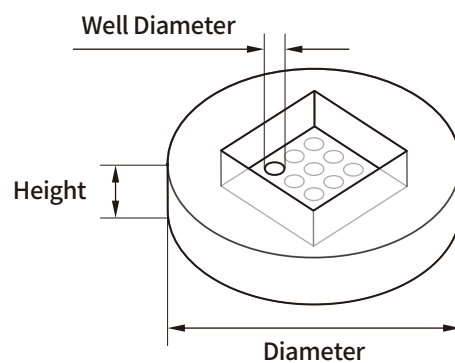


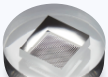
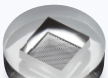
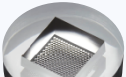
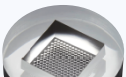
11 Harvest the spheroids into an E-tube or conical tube.

StemFIT 3D® Specification

Dimensions

- H1613200, C100600 : D22 x H10 (mm)
- H853400, H389600L, H389600H : D30 x H15 (mm)
- H449800, H2951000, C163000, C253000 : D40 x H20 (mm)



	Well Type	No. of Wells	Well Diameter	Amount of Cells	Volume of Seeding Medium	Volume of Total Medium
 H1613200	Hexagon	1,613	200 µm	$\geq 6.0 \times 10^5$	500 µl	1 ml
 H853400	Hexagon	853	400 µm	$\geq 1.2 \times 10^6$	1 ml	1.5 ml
 H389600L H389600H	Hexagon	389	600 µm	$\geq 1.2 \times 10^6$	1 ml	(L) 1.3 ml
						(H) 1.7 ml
 H449800	Hexagon	449	800 µm	$\geq 2.4 \times 10^6$	2 ml	4 ml
 H2951000	Hexagon	295	1,000 µm	$\geq 2.4 \times 10^6$	2 ml	4 ml
 C100600	Circle	100	600 µm	$\geq 6.0 \times 10^5$	500 µl	700 µl
 C163000	Circle	16	3,000 µm	2.5×10^5 / well	20 µl / well	5 ml
 C253000	Circle	25	3,000 µm	2.5×10^5 / well	20 µl / well	3 ml

For organoids, the cell number may vary from the values suggested in this protocol.