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3D Insert™ Cell Seeding Protocol

Application

This is the protocol for cell seeding cells on 3D Insert™ scaffolds in a **non-treated** cell culture plate/dish. **It is critical to follow this 3D cell seeding protocol. Following this protocol will allow the cell suspension to be completely held inside the scaffold by the capillary force so that the cells will attach to the scaffolds.**

Materials and Equipment

Sterile ***non-treated*** cell culture plate/dish
Pipette
Vacuum
Cell culture incubator
Sterile forceps
Sterile pipette tips
Biosafety hood

Procedure

1. Calculate the appropriate cell concentration for 3D cell seeding. Refer to **Tables III and IV** for the 3D scaffold's cell growth areas. We suggest using the same cell seeding density (number of cells/cm^2) as you normally use with 2D cell culture. See the Appendix for an example.

2. Remove cells of interest from culture and resuspend in culture medium at an appropriate concentration.

3. Slowly pipette the correct volume (**Table I**) of this cell suspension onto the top surface of each 3D Insert™ scaffold, covering 80-90% of the scaffold's surface. **Note: Do not allow the cell suspension to contact the sides of the wells. This will result in a reduced seeding efficiency**.

Table I

Scaffold Size	Seeding Volume (µl)			
	PS1520	PS3040	PCL3030	PCL3050
96-well	15	20	20	25
48-well	25	50	50	70
24-well	60	120	130	160
12-well	120	240	270	340
6-well	300	600	690	880
100-mm Dish	1850	N/A	4300	N/A

4. Gently place plates into incubator. Avoid agitating the plates/dish!

5. After 3 h, remove the plates/dish containing the scaffolds from the incubator. Add the appropriate volume (**Table II**) of fresh medium into each well of the plate/dish in a biosafety hood. Make sure that the scaffolds are completely immersed and sitting at the bottom of the wells. Return the plate/dish to the incubator.

Table II

Scaffold Size	Volume (µl) To Add After 3 h				Total Volume (µl) After 3 h			
	PS1520	PS3040	PCL3030	PCL3050	PS1520	PS3040	PCL3030	PCL3050
96-well	185	180	180	175	200	200	200	200
48-well	225	200	200	180	250	250	250	250
24-well	440	380	370	340	500	500	500	500
12-well	1380	1260	1230	1160	1500	1500	1500	1500
6-well	1700	1400	1310	1120	2000	2000	2000	2000
100-mm Dish	13150	N/A	10700	N/A	15000	N/A	15000	N/A

6. Continue with normal cell tissue culture until analysis.

Table III: Surface Area for Cell Seeding and Conversion Ratio from 2D to 3D PS Scaffolds

Scaffold Size	2D Growth Area	3D Growth Area (PS1520)	3D Growth Area (PS3040)	3D/2D Ratio (PS1520)	3D/2D Ratio (PS3040)
96-well	0.32 cm^2	1.36 cm^2	1.21 cm^2	4.2	3.8
48-well	1.00 cm^2	4.28 cm^2	3.78 cm^2	4.3	3.8
24-well	1.90 cm^2	10.20 cm^2	9.56 cm^2	5.4	5.0
12-well	4.00 cm^2	21.08 cm^2	19.65 cm^2	5.3	4.9
6-well	9.60 cm^2	54.02 cm^2	52.10 cm^2	5.6	5.4
100-mm Dish	56.75 cm^2	346.67 cm^2	N/A	6.1	N/A

Table IV: Surface Area for Cell Seeding and Conversion Ratio from 2D to 3D PCL Scaffolds

Scaffold Size	2D Growth Area	3D Growth Area (PCL3030)	3D Growth Area (PCL3050)	3D/2D Ratio (PCL3030)	3D/2D Ratio (PCL3050)
96-well	0.32 cm^2	2.03 cm^2	1.53 cm^2	6.3	4.8
48-well	1.00 cm^2	7.74 cm^2	6.08 cm^2	7.7	6.1
24-well	1.90 cm^2	18.28 cm^2	13.74 cm^2	9.6	7.2
12-well	4.00 cm^2	39.27 cm^2	27.90 cm^2	9.8	7.0
6-well	9.60 cm^2	99.21 cm^2	75.62 cm^2	10.3	7.9
100-mm Dish	56.75 cm^2	616.35 cm^2	N/A	10.9	N/A

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APPENDIX

An Example of How to Calculate The Cell Suspension Concentration for 3D Scaffolds Seeding

We suggest using the same cell seeding density (number of cells/cm^2) as you normally use with 2D cell culture. However, because the 3D Insert™ provides a much higher surface area (**Tables III-IV**) than a traditional 2D well, you will need to seed more cells in each scaffold than you normally would in a 2D well. Below is a 3D cell seeding example for seeding cells on a total of 24 scaffolds in a 96-well package of 3D Insert™–PS152096-24.

a. Assume you will need to seed 1,000 cells in each well of a 96-well plate when you perform 2D cell culture. When conducting your experiment using the 3D Insert™–PS152096-24, you will need to seed 1,000 x 4.2 = 4,200 cells in each 3D scaffold, because the surface area of the 96-well 3D Insert™–PS152096-24 is 4.2 times greater (the factor in column 5 of **Table III**) than the surface area of a traditional 2D, 96-sized well. **NOTE: Be sure to use the appropriate ratio listed in Tables III and IV for your experiment.**

b. These 4,200 cells will be in a medium volume of 15 µl (the seeding volume for a 96-well 3D Insert™–PS152096-24). Thus, the cell suspension concentration you need to prepare will be 4,200/0.015 ml = 280,000 cells/ml.

c. A normal 3D Insert™–PS152096-24 package contains 24 scaffolds. For an experiment using all of these scaffolds at a concentration of 280,000 cells/ml, you will need a total cell suspension of 24 x 0.015 ml = 0.36 ml to seed all 24 scaffolds. Be sure to prepare more cell suspension in order to compensate for possible pipette loss. We typically prepare an extra volume for two extra scaffolds. In this case, the extra volume is 2 x 0.015 ml = 0.03 ml. Therefore, the total cell suspension volume for seeding is 0.36 ml + 0.03 ml = 0.39 ml.

d. Using your original/stock cell suspension, make a total 0.39 ml of 280,000 cells/ml cell suspension. This is the working volume and concentration necessary to seed 24 3D Insert™–PS152096-24 scaffolds.

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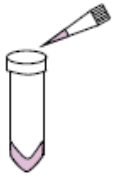
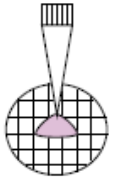
3D Biotek Cell Seeding Flow-Chart

1. Unpack scaffolds in tissue culture hood

2. Pipette the appropriate volume of cell suspension (Refer to chart below and to 3D cell seeding protocol)



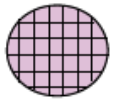
3. Carefully place suspension droplet onto the center of the scaffold surface



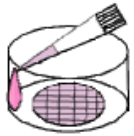
4. Immediately after seeding: Cell droplet will cover ~80-90% of scaffold

3 h incubation
37° C, 5% CO_2

5. Cell droplet will penetrate entire scaffold



6. Gently add remaining media along well's edge (Refer to chart below)



7. Perform experiments
- Monitor cell growth and morphology in real-time with an inverted light microscope
- Any assay can be easily performed with 3D Insert™ scaffolds with little to no modification!

Scaffold Size	Seeding Volume (µl)			
	PS1520	PS3040	PCL3030	PCL3050
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6-well	300	600	690	880
100-mm Dish	1850	N/A	4300	N/A

WARNING:
DO NOT touch scaffold with pipette tip!
DO NOT let droplet touch sides of wells. This will decrease seeding efficiency!

Scaffold Size	Volume to Add After 3h (µl)			
	PS1520	PS3040	PCL3030	PCL3050
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