microtissues®
natural 3D

Casting, Equilibrating and Seeding the 3D Petri Dish®

1. Rinse micro-molds for casting 3D Petri Dishes® in dH_2O.
2. Place micro-molds for casting 3D Petri Dishes® in container suitable for autoclave sterilization.
3. Measure 1g of high quality pure agarose powder and place into a dry 100 ml autoclave-safe glass bottle. Be sure bottle and agarose powder are dry with no liquid or moisture. Screw on lid so that it is secure, but still loose.
4. Autoclave micro-molds for casting 3D Petri Dishes® and bottle with agarose powder for 30 minutes on dry cycle.
5. In a biosafety cabinet using aseptic technique, add 50 ml of sterile saline [0.9% (w/v) NaCl] to the bottle containing sterilized agarose powder. Screw on lid so that it is secure, but still loose. Swirl bottle to mix agarose powder.
6. Use microwave oven to boil and completely dissolve the agarose powder. Stop microwave every 10 seconds and swirl bottle to help dissolve the agarose. Be sure there are no small bits of translucent undissolved agarose.

   CAUTION: Molten agarose is very hot, can boil over when the bottle is swirled and can cause severe skin burns. Always use appropriate personal protection (gloves, oven mitt, eye protection and lab coat) when working with molten agarose.
7. Allow molten agarose to cool to about 60-70°C. In a biosafety cabinet using aseptic technique, pipette 500 µl of molten agarose into a **12-series** micro-mold or 330 µl into a **24-series** micro-mold. Avoid creating bubbles while mixing or pipetting agarose.
8. Remove any small bubbles that may be trapped in the small features of the micro-mold by pipetting or gentle scrapping before the agarose gels.
9. After the agarose has gelled, carefully flex the micro-mold to remove the 3D Petri Dish®. Do not over flex the micro-mold as it can crack the 3D Petri Dish®. Some practice may be necessary to remove the 3D Petri Dish® from the micro-mold. Transfer 3D Petri Dish® to a 12 well or 24 well tissue culture plate.
10. To equilibrate the 3D Petri Dish®, add cell culture medium (2.5ml/well for 12 well plate and 1.0ml/well for 24 well plate). Incubate for 15 minutes or longer. Remove culture medium and replace with fresh medium. Repeat once more to equilibrate the 3D Petri Dish® with culture medium.
11. Trypsinize cells, count and prepare the desired cell seeding number in the specified volume for the 3D Petri Dish® of choice (see **Tables 1-4**).
12. Remove culture medium surrounding outside of the 3D Petri Dish® and tilt the tissue culture plate so that the medium from the cell seeding chamber of the 3D Petri Dish® can also be carefully removed.

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13. Carefully seed cell suspension drop wise into the cell seeding chamber.

14. Allow ~10 minutes for cells to settle into the features of the 3D Petri Dish®. Then add additional medium to the outside of the 3D Petri Dish® (2.5ml/well for 12 well plate and 1.0ml/well for 24 well plate).

15. Place the tissue culture plate in the cell culture incubator and exchange medium surrounding 3D Petri Dish® as needed.

Table 1. Specifications of the 3D Petri Dishes® and the micro-molds used to cast them.

Catalogue #	Features	Number of features	Array	Agarose volume	Cell seeding volume
12-256	Small spheroids	256	16 x 16	500 µl	190 µl
12-81	Larger spheroids	81	9 x 9	500 µl	190 µl
12-60TR	Troughs	60	4 x 15	500 µl	190 µl
12-36TO	Toroids	36	6 x 6	500 µl	190 µl
24-96	Small spheroids	96	8 x 12	330 µl	75 µl
24-35	Larger spheroids	35	5 x 7	330 µl	75 µl
24-24TR	Troughs	24	3 x 8	330 µl	70 µl
24-H	Honeycomb	1	1	330 µl	20 µl

Table 2. Estimates of cell seeding numbers for smaller spheroids in the 3D Petri Dish®.		**# 12-256**	**# 24-96**
		12 well plate Small spheroids: 256	24 well plate Small spheroids: 96
***Nominal spheroid diameter (µm)**	**~Cells/spheroid**	**Total cells seeded (cells/190 µl)**	**Total cells seeded (cells/75 µl)**
50	15	3,840/190 µl	1,440/75 µl
100	125	32,000/190 µl	12,000/75 µl
150	421	107,000/190 µl	40,000/75 µl
200	1,000	256,000/190 µl	96,000/75 µl
250	1,953	500,000/190 µl	187,000/75 µl
300	3,375	860,000/190 µl	324,000/75 µl

*These are estimates only. Estimates of spheroid diameter are based on the following assumptions, i.) diameter of input cells is 20µm (4,180µm^3), ii.) total spheroid volume equals cell volume times the number of input cells, iii.) the spheroid is a perfect sphere and, iv.) cells are perfectly distributed to all recesses. In practice, spheroid size and shape will vary depending on cell type.

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Table 3. Estimates of cell seeding numbers for larger spheroids in the 3D Petri Dish®.		#12-81	#24-35
		12 well plate Larger spheroids: 81	24 well plate Larger spheroids: 35
***Nominal spheroid diameter (μm)**	**~Cells/spheroid**	**Total cells seeded (cells/190 μl)**	**Total cells seeded (cells/75 μl)**
200	1,000	81,000 /190 μl	35,000/75 μl
300	3,375	273,000/190 μl	118,000/75 μl
400	8,000	648,000/190 μl	280,000/75 μl
500	15,625	1.3 x10^6/190 μl	547,000/75 μl
600	27,000	2.2 x10^6/190 μl	945,000/75 μl
700	42,875	3.5 x10^6/190 μl	1.5 x10^6/75 μl

* These are estimates only. Estimates of spheroid diameter are based on the following assumptions, i.) diameter of input cells is 20μm (4,180μm^3), ii.) total spheroid volume equals cell volume times the number of input cells, iii.) the spheroid is a perfect sphere and, iv.) cells are perfectly distributed to all recesses. In practice, spheroid size and shape will vary depending on cell type.

Table 4. Estimates of cell seeding numbers for making microtissues with complex shapes in the 3D Petri Dish®.

Catalogue #	Feature: #	Seeding volume	~Cells/ feature	*Total cells seeded
12-60TR	Troughs: 60	190 μl	20,000	1.2 x10^6/190 μl
24-24TR	Troughs: 24	70 μl	20,000	480,000/75 μl
12-36TO	Toroids: 36	190 μl	20,000	720,000/190 μl
24-H	Honeycomb: 1	20 μl	140,000	140,000/20 μl

*These cell seeding densities are suggestions for initial experiments. Results can vary with cell type, so a range of cell seeding densities should be tested.

Care and maintenance of micro-molds for casting 3D Petri Dishes®.

When not in use, store micro-molds for casting 3D Petri Dishes® in a dust and fibre-free environment. Periodically examine micro-molds under a dissecting microscope to be sure small features are free of dust and small fibres.

3D Petri Dishes® and the micro-molds used to cast them are sold and distributed for evaluation and/or research purposes only, and are not intended for manufacturing, re-sale, human or animal diagnostic or therapeutic use.

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