Obatala Sciences' Protocol 306
How Do I Create Spheroids in ObaGel® Cultures?

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Reagents, Materials, and Equipment

- Obatala Sciences' ObaGel® (Catalog #OS-301)
- Obatala Sciences' ObaVate™ (Catalog #OS-302)
- Obatala Sciences' Human Adipose-Derived Stromal/Stem Cells (Catalog #OS-101), Human Stromal Vascular Fraction Cells (Catalog # OS-107-01), or equivalent cryopreserved primary cell product
- Obatala Sciences' StromaQual™ Stromal Medium (Catalog #OS-001) or medium of choice
- Viability stains such as trypan blue or acridine orange/propidium iodide
- 0.05% Trypsin/EDTA
- T150 or T175 flask
- 70% ethanol
- Phosphate buffer saline (1X) or equivalent product
- Sterile paper towel or kimwipe
- Conical centrifuge tube
- Micropipette and tips
- 100 mm x 15 mm Petri dish(es)
- Benchtop centrifuge
- 37°C, 5% CO_2 incubator
- BSL2 Biological Safety Cabinet
- Wet ice for prolonged handling

Protocol

Initial Handling of Your Obatala Sciences' Products

1. When you receive the package containing your ObaGel® and ObaVate™ products, they may arrive on wet ice or dry ice depending on the shipping conditions.
    a. Prior to use, thaw the unopened products overnight at 4°C until completely thawed. Do not thaw at room temperature or attempt to warm products at higher temperatures.
    b. Aliquot necessary volumes for immediate use into separate containers to avoid repeated freeze/thaw cycles.

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c. After thawing, ObaGel® can be stored at 4°C for <48 hours prior to use. For longer term storage up to 3 months, store at -20°C. For storage >3 months, store at -80°C (shelf life 1 year).
d. After thawing, ObaVate™ can be stored at 4°C for up to 4 weeks, <3 months at -20°C, and up to 2 years at -80°C.

2. After thawing, you may notice protein precipitant present in ObaGel®. This is normal and does not impact function or quality of the product. In fact, it is beneficial to the formation of 3D constructs!

Note: Do not attempt to spin or otherwise remove the precipitant from the product, as it will clump and aggregate. Unaltered, the precipitant will disperse when pipetting.

Harvesting Adherent Cells (Adapted from **Protocol 102 – How to Harvest Adherent Cells**)

1. From a confluent T150 or T175 flask, aspirate medium and wash with warm, sterile 1X PBS.
2. Aspirate the PBS, add 0.05% Trypsin/EDTA, and place in the incubator at 37°C, 5% CO_2 for 5 minutes.
3. Add equal volume of StromaQual™ to the flask to neutralize the trypsin.
4. Transfer entire volume to a 50 mL conical tube.
5. Using an automated cell counter, hemacytometer, or other appropriate cell counting technique, determine the relative percentage of live cells and dead cells to determine total live cell count using a viability stain such as trypan blue or acridine orange/propidium iodide.
6. Seal the cap on the conical tube. Transfer the conical tube to the bench top centrifuge and spin at 300 x g (1200 rpm) for 5 minutes at room temperature.
7. Retrieve ObaGel® and ObaVate™ from 4°C or ice bucket.
8. Return the conical tube to the biological safety cabinet and observe that a distinct and intact pellet is retrieved. Carefully aspirate the supernatant from the cell pellet.
9. Resuspend the cell pellet in one-part ObaGel® to three parts ObaVate™ for a final concentration of 2 x 10^6 cells per mL.

Spheroid Formation in ObaGel® using the Hanging Drop Method

1. Add 10-15 mL warm, sterile 1X PBS to a petri dish.
2. Remove the lid of a petri dish and lay it upside down with the inside facing upright.
3. Using a multichannel or single channel pipet, place 27 µL drops onto the lid of the petri dish, making 4 rows of 8 and 2 rows of 6.
    1. It is ideal to have ~30 to 60 drops per dish, depending on the size of the petri dish.
4. Carefully flip the lid right side up and place it back onto the petri dish.
5. Label the lid and place at 37°C, 5% CO_2 incubator for 24-48 hours.
6. To visualize in the microscope, replace the bottom of the petri dish with a new one, invert the plate, and visualize under a microscope at 4X and 10X objectives.

Recommended Protocols

Obatala Sciences' Protocol 101–How Do I Thaw Cryovials of Cells from Obatala Sciences?
Obatala Sciences' Protocol 102–How Do I Harvest Adherent Cells from Obatala Sciences?
Obatala Sciences' Protocol 301–How Do I Create 3D Cultures with ObaGel®?

We expect that you will have new ideas on how to use our product that extend beyond these boundaries and look forward to hearing about novel ways you can use ObaGel® in your discovery research. Please share your findings with us when they become available.

Remember, any laboratory that mentions Obatala Sciences' products by name in a publication is eligible for a 10% discount on their next order! We appreciate not only your business but your endorsement of our products!

Appendix A: Generation of Spheroids in ObaGel® Workflow

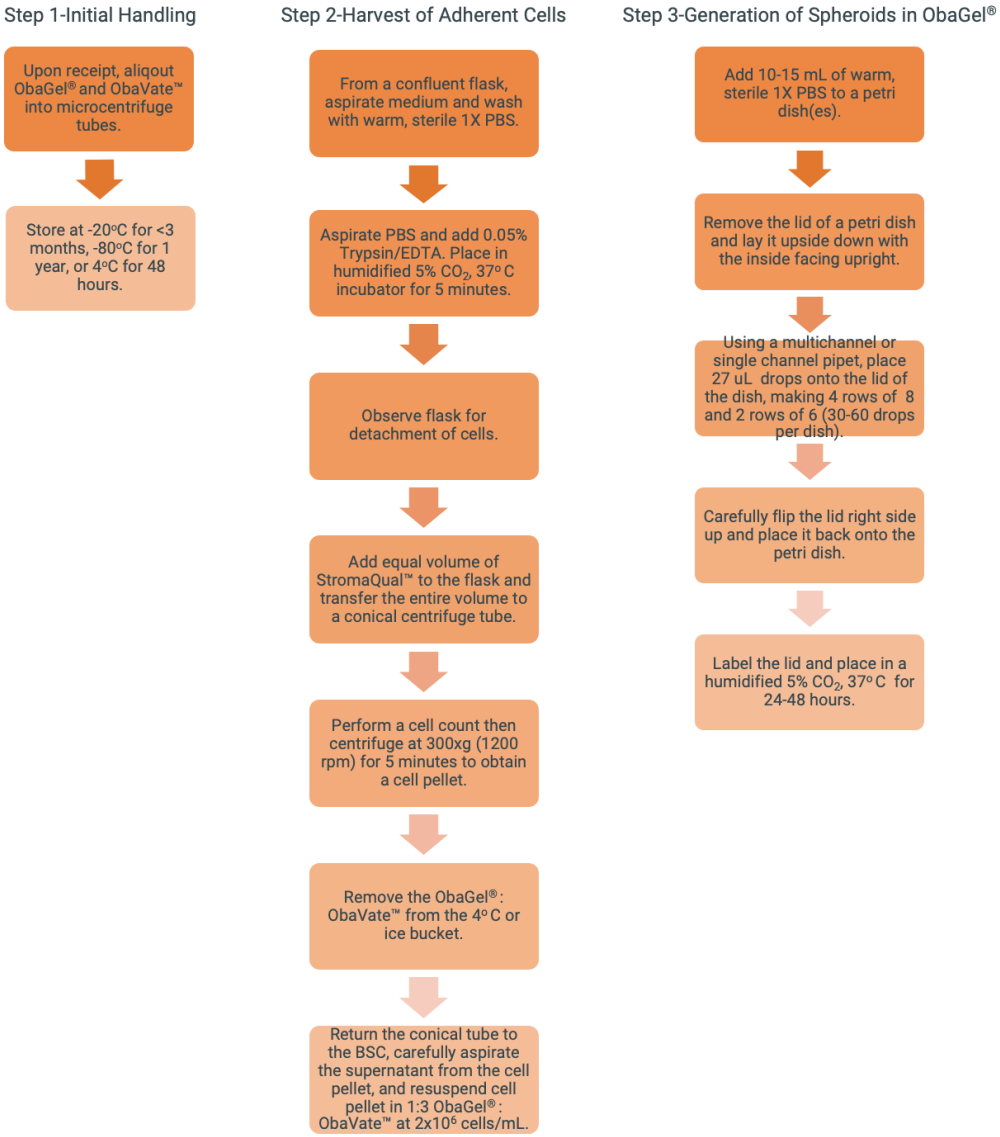

Figure 1. Workflow diagram outlining the steps required spheroid and organoid formation using ObaGel®. Steps for establishing spheroids and organoids in ObaGel® include: Initial handling of ObaGel®, harvesting of cells, and establishing spheroids and organoids in ObaGel®.

Appendix B: Troubleshooting

Problem	Reason	Solution
No gelation observed	ObaGel® pre-warmed at 37°C or equivalent prior to use	Do not heat ObaGel® prior to use, maintain on ice during use
	Repeated freeze-thaws	Aliquot ObaGel® into smaller volumes and thaw as needed
Spheroids do not remain intact	Insufficient incubation period	Allow the petri dishes to remain at 37°C, 5% CO_2 for 24-48 hrs prior to manipulation