



## Obatala Sciences™ Protocol 302 How Do I Create 2D Cultures with ObaGel®?

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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) or equivalent cryopreserved primary cell product
- ◆ Obatala Sciences™ StromaQual™ Stromal Medium (Catalog #OS-001) or medium of choice
- ◆ Obatala Sciences™ Live/Dead Assay Medium (Catalog #OS-008-01)
- ◆ 70% ethanol
- ◆ Sterile paper towel or kimwipe
- ◆ Conical centrifuge tube
- ◆ Culture plate & micropipette for desired format, protocol is optimized for 150 mm plate or a T150 flask
- ◆ Wet ice for prolonged handling

### Calculations

Reagent	150mm plate	T150 flask	6-well plate
ObaGel	0.5 mL	0.5 mL	0.5 mL/well
ObaVate	0.5 mL	0.5 mL	0.5 mL/well
StromaQual	35 mL	35 mL	5 mL
hSVF cells	5-30 x 10 <sup>3</sup> cells/cm <sup>2</sup>	5-30 x 10 <sup>3</sup> cells/cm <sup>2</sup>	5-30 x 10 <sup>3</sup> cells/cm <sup>2</sup>
hASC cells	50-300 x 10 <sup>3</sup> cells/cm <sup>2</sup>	50-300 x 10 <sup>3</sup> cells/cm <sup>2</sup>	50-300 x 10 <sup>3</sup> cells/cm <sup>2</sup>

### 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.  
(Note: If you ordered your ObaGel® to arrive in a cold pack (4° C equivalent), it will arrive already thawed.)
  - 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.
  - c. After thawing, product 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).
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!

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.

### Seeding Cells on ObaGel® Coated Plates for 2-Dimensional Culture

1. Thaw ObaGel® and ObaVate™ overnight at 4 °C until completely thawed and no ice crystals remain in solution (see handling instructions above). Keep refrigerated or on wet ice prior to use.  
*(Note: What could I do wrong at this step? The ObaGel® product is temperature sensitive. If you were to immediately warm it up to 37° C, you will inactivate its gelling properties. Therefore, do not allow the thawed product's temperature to exceed refrigerator temperature (4°C) during the thawing process.)*
2. Place the aliquots of ObaGel® and ObaVate™ on wet ice and transfer to a BSL2 biological safety cabinet. Transfer the recommended volume of ObaGel® to a sterile centrifuge conical tube and add an equal volume of ObaVate™. Mix the reagents thoroughly by pipetting.
3. Transfer 1mL of the ObaGel®-ObaVate™ solution to the culture surface. Tilt to coat the entire surface of the plate or flask.
4. Maintain the plate in the biological safety cabinet for at least 15 minutes at room temperature.
5. After 15 minutes, tilt the plate or flask and observe whether any excess volume collects at the periphery of the surface. Use a micropipette to remove any excess volume, pipetting slowly at the periphery to reduce shear stress on the coating surface.
6. Plate Obatala Sciences™ Human Adipose-Derived Stromal/Stem Cells (Catalog #OS-101) or equivalent cryopreserved primary cell product at a concentration of 5 to 30 X 10<sup>3</sup> cells/cm<sup>2</sup> in the recommended volume of Obatala StromaQual™ Stromal Medium (Catalog #OS-001)
7. Transfer the plate or flask to a humidified 5% CO<sub>2</sub> incubator at 37°C.
8. Leave the flask overnight in the humidified 37°C incubator. Evaluate for cell adhesion under phase contrast microscopy after at least a 24 hr incubation period. Proceed to feed and maintain as appropriate for the cell of interest.

## 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 103 – How Do I Cryopreserve Culture-Expanded Cells from Obatala Sciences™?

After you have established your 2-dimensional cultures, you can proceed to your next planned experimental endpoints, which might include but are by no means limited to proliferation, differentiation, or implantation into recipient mice or animal models in vivo.

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!*