# Obatala Sciences™ Protocol 306
# How Do I Create ObaGel® Cultures for *In Vivo* Implantation?

Written by: Obatala Sciences™ Staff
Last Updated: April 2022

## 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
- AdipoQual3D™ Adipogenic Differentiation Medium (Catalog #OS-310)
- Obatala Sciences™ Live/Dead Assay Medium (Catalog #OS-008-01) or Trypan Blue Exclusion Stain (Catalog #OS-011-01)
- 70% ethanol
- Sterile paper towel or kimwipe
- Conical centrifuge tube
- Micropipet and tips
- Well plate & micropipette for desired format, protocol is optimized for 24-well or 96-well plate
- Wet ice for prolonged handling

## Protocol

Initial Handling of Your Obatala Sciences™ Products

1. Prior to use, thaw ObaGel® and ObaVate™ overnight at 4°C until completely thawed. Do not thaw at room temperature or attempt to warm products at higher temperatures.
2. Aliquot necessary volumes for immediate use into separate containers to avoid repeated freeze/thaw cycles.
3. After thawing the products 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 of 1 year).
4. After thawing, protein precipitant may be observed in ObaGel®. This is normal and does not impact function or quality of the product. If this occurs, ensure that the lid is secured tightly on the bottle and shake the ObaGel® product three to four times to mix the product thoroughly before use.

Obatala Sciences, Inc.
2000 Lakeshore Dr. #4020
New Orleans, LA 70148
504-300-0266
www.obatalasciences.com

5. Do not attempt to centrifuge or use other means to remove precipitant from the product as it will clump and aggregate. Unaltered, the precipitant will disperse when pipetting.

Seeding Cells on ObaGel® Coated Plates for 3-Dimensional Culture

1. Thaw ObaGel® and ObaVate™ overnight at 4°C until completely thawed and no ice crystals remain in solution. Keep refrigerated or on wet ice prior to immediate use.
2. Remove cryopreserved vial(s) of hASCs, hSVF, or other preferred cell type from cryogenic storage conditions according Obatala's SOP. Thaw the vial(s) of cells after removal from cryogenic storage until the ice crystals are no longer visible in the vial.
3. Once thawed, rinse the external surface of the vial(s) with 70% ethanol and dry with a sterile paper towel or Kim wipe. Transfer the cryovial to a BSL2 biological safety cabinet.
4. Inside the biological safety cabinet, carefully open the cryovial. Transfer the contents of the vial to a 50 mL conical tube using a micropipette. Adding dropwise, dilute contents of the vial slowly by adding 4 mL of StromaQual™. Pipette cell suspension multiple times to homogenize cells in solution.
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
    a. For hSVF, use Live/Dead Assay Medium or Ethidium Bromide/Acridine Orange solution.
    b. For hASCs, use Trypan Blue Exclusion Stain
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 required volume of ObaVate™ at a concentration of at least 5 x $10^5$ cells per mL. A final concentration of 1x$10^6$ hSVF cells/mL or 1x$10^5$ hASC cells/mL is recommended (see calculations table in Section 8.)
10. Add one-part ObaGel® to three parts ObaVate™/cell mixture (1:3 ratio) and pipette several times to mix well.
    a. Once components are added, work quickly to avoid cells sinking or clumping in solution. Mix between additions.
    b. Keep suspension on ice for a prolonged seeding period (>15 minutes).
11. Aliquot ObaGel®/ ObaVate™ cell suspension into well plate, mixing thoroughly and regularly between replicates.
    a. See calculations table in **Protocol 301** for recommended volumes.
    b. As a starting point, we recommended aliquoting 1 mL of mixture to the center of a single well of a 24 well plate; variations on this volume can be implemented based on smaller or larger well sizes at your discretion and need.

12. After aliquoting, cell: ObaGel® mixture can be prepared immediately for implantation or seeded into multi-well plates for 3D cell growth, priming, and/or differentiation prior to implantation. If formation of a 3D structure is required prior to implantation, transfer the plate to a humidified 5% $CO_2$ incubator at 37C. Observe after 1 hour for initial signs of gelation, and again at 24 hours for consistent gelation.
    a. Gelation can be observed when tilting the plate and observing that a layer of gel is present at the top of the well when oriented at an angle.

Maintenance of 3-Dimensional ObaGel® cultures

1. Feed the 3-dimensional constructs once per week (every 7 days) with 25% of the initial seeding volume using StromaQual3D™ medium.
    a. For adipogenic differentiation of the cultures, follow the same procedure in 9.3.1 using AdipoQual3D™ medium
2. Remove the conditioned or spent media from each well by tilting the plate and using a micropipette to remove liquid that collects at the bottom region of the well with the pipette tip.
    a. When removing media from the wells, be careful not to disturb the 3-dimensional construct. Remove volume slowly and with consistent speed as to not draw up the gel from below the liquid interface.
    b. Proceed with feedings and do not allow the 3D constructs to dry out after removing conditioned medium.
3. Replace conditioned media with StromaQual3D™ medium for maintenance of 3D cultures.
    a. Pipet a new volume of medium against the wall of the well to reduce any shear stress to the 3-dimensional construct when the liquid flows onto the gel interface.

Implantation of 3-Dimensional ObaGel® constructs

1. When transporting ObaGel® constructs to the vivarium, do not place on ice. Wrap plates in aluminum foil or appropriately insulated material to aid in temperature maintenance. Place inside a Styrofoam container.
2. After anesthetizing the mice, prepare for injections.
3. Aspirate ObaGel® constructs from the individual wells into syringes by removing the needle from the syringe first, aspirating the ObaGel® construct, and finally twisting the needle back onto the syringe for injection.
4. Immediately inject the ObaGel® constructs into the dorsal subcutaneous top, bottom, left, and right locations (4 locations, 4 injections per mouse).

## 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™?
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!*