# Obatala Sciences™ Protocol 301
# How Do I Create 3D Cultures with ObaGel®?

Written by: Obatala Sciences™ Scientific Team
Last Updated: August 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) or equivalent cryopreserved primary cell product
- Obatala Sciences™ StromaQual™ Stromal Medium (Catalog #OS-001) or growth medium of choice
- Obatala Sciences™ StromaQual3D™ Stromal Medium (Catalog #OS-309) or 3D growth medium of choice
- Obatala Sciences™ AdipoQual3D™ Adipogenic Differentiation Medium (Catalog # OS-310) or 3D differentiation medium of choice
- Obatala Sciences™ Live/Dead Assay Medium (Catalog #OS-007)
- 70% ethanol
- Sterile paper towel or kimwipe
- Conical centrifuge tube
- Well plate & micropipette for desired format
- Wet ice for prolonged handling

## Calculations

| ***Reagent*** | ***6-well (5mL)*** | ***24-well (1mL)*** | ***96-well (100uL)*** |
|---|---|---|---|
| *ObaGel®* | 1.25mL/well | 250uL/well | 25uL/well |
| *ObaVate™* | 3.75mL/well | 750uL/well | 75uL/well |
| *hSVF cells* | 5,000,000 cells/well | 1,000,000 cells/well | 100,000 cells/well |
| *hASC cells* | 500,000 cells/well | 100,000 cells/well | 10,000 cells/well |
| *3D Medium (feedings)* | 1.25mL/well | 250uL/well | 25uL/well |

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

## General Requirements

1. All personnel should be trained and certified by the Principal Investigator regarding Universal Precautions and Handling of Bloodborne Pathogens.
2. All procedures should be conducted by investigators using appropriate personal protective equipment at all times. Any waste materials should be decontaminated (bleached) and disposed of using appropriate biohazard waste containers.
3. Wear protective eyewear during handling of cryovial(s).

## 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!
   a. 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.
3. Obatala Sciences™ 3D media products (StromaQual3D™, AdipoQual3D™) will arrive on cold packs (4°C equivalent)
   a. Store at 4°C upon arrival
   b. Prior to use, take an aliquot of media and place on the benchtop for 5-10 minutes to warm to room temperature. Do not attempt to warm products at higher temperatures (i.e., in a water bath)

Seeding Cells in ObaGel® for 3-Dimensional Cultures:

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. Remove cryopreserved vial(s) of human adipose derived stromal vascular fraction cells (Obatala Science's cells hSVF or hASC contain$10^6$ cells per ml) from cryogenic storage conditions according to your laboratory's standard operating procedure. Thaw the vial of cells after removal from cryogenic storage until the moment ice crystals disappear from the contents.
   *(Note: What could possibly go wrong? The worst-case scenario is that liquid nitrogen has leaked inside of the cryovial containing the cells. While the cryovial design used at Obatala Sciences™ are selected specifically to reduce any risk of having this happen, if it does, the expanding nitrogen gas within the vial can cause the vial to explode. That's why you need to wear eye protection, gloves and lab coat!)*
3. Once thawed, rinse the external surface of the vial with 70% ethanol and dry with a sterile paper towel or kimwipe. 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 conical centrifuge tube using a micropipette. Adding dropwise, dilute the contents of the vial slowly by adding of 4 ml of Obatala Sciences™ StromaQual™ Stromal Medium (Catalog #OS-001) or medium of choice. Pipette cell suspension multiple times to disperse cells and break up any tissue fragments.
   *(Note: Why do I need to add the Obatala StromaQual™ Stromal Medium dropwise? We cryopreserve the cells in the presence of a cryoprotective agent. If we dilute the concentration of the cryoprotective agent too fast, the cells cannot equilibrate the small molecule across their membranes. When that happens, the recovered cells are more likely to display a low viability. So, to keep your cells happy, do not dilute them too fast!)*
5. According to your laboratory's standard operating procedures, determine the relative percentage of live cells and dead cells to determine total live cells and viability (%).
   a. A hemocytometer or automatic cell counter may be used
   b. For hSVF cells, we recommend Obatala Sciences™ Live/Dead Assay Medium (Catalog #OS-008-01) for viability stain.
   c. For hASC, we recommend trypan blue viability stain.
6. Seal the cap on the centrifuge tube. Transfer the centrifuge tube to a bench top centrifuge or equivalent and centrifuge at 300x g (1200 rpm) for 5 minutes at room temperature.
7. Retrieve ObaGel® and ObaVate™ from 4ºC refrigerator or ice bath.
8. Return the centrifuged tube to the biological safety cabinet and observe that a distinct and intact pellet has been 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 $5x10^5$ cells per mL.

a. We recommend a final concentration of $1x10^6$ hSVF cells/mL or $1x10^5$ hASC cells/mL (see calculations table)

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 culture plate, mixing thoroughly and regularly between replicates.
    a. See calculations table above for recommended volumes.
    b. As a starting point, we recommend aliquoting 1 mL of the 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, transfer the plate to a humidified 5% $CO_2$ incubator at 37º C. Observe after 1hr for initial signs of gelation, and again after 24hr 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. Similarly, when tapping the plate gently under a brightfield microscope the well contents do not appear liquid and cells appear to "jiggle" in suspension.

    *(Note: Why do I need to transfer the ObaGel® to the incubator? The biomechanical properties of ObaGel® change as a function of temperature and cell metabolism. The cells release enzymes that process the ObaGel® proteins and in the presence of the activation agent, cross linking occurs which contributes to a 3-dimensional architecture. The cell metabolism is optimal at 37º C within the incubator.)*
13. Feeding and maintaining the 3-dimensional ObaGel® cultures:
    a. We recommend feeding the 3-dimensional constructs once per week (every 7 days) with 25% of the initial seeding volume. The intent is to replace medium that has evaporated from the culture over time while maintaining the original plating volume.
        i. For maintenance of 3D cultures, we recommend Obatala Sciences™ StromaQual3D™ medium
        ii. For adipogenic differentiation of 3D cultures, we recommend Obatala Sciences™ AdipoQual3D™ medium
        iii. When using Obatala Sciences™ 3D media products, do not use any methods to heat the products (i.e., in a water bath). We recommend warming an aliquot of media at room temperature on the benchtop for 5-10 minutes prior to use.
    b. Remove the conditioned or spent media from each well by tilting the plate and using a micropipette to remove the liquid that collects at the bottom

region of the well, taking care not to touch the actual base of the well with the pipette tip.
    i. When removing media from the well, 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.
    ii. Proceed with feedings and do not allow the 3D constructs to dry out after removing conditioned medium.
c. Replace conditioned media with StromaQual3D™ for maintenance of 3D cultures or AdipoQual3D™ for differentiation of cultures.
    i. We recommend pipetting the 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.

After you have established your 3-dimensional constructs, you can proceed to your next planned experimental endpoints, which might include but are by no means limited to proliferation, differentiation, flow cytometry, or implantation into recipient mice or animal models in vivo.

We expect that you will have new ideas on how to use our products that extend beyond these boundaries and look forward to hearing about novel ways you can use them 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!*