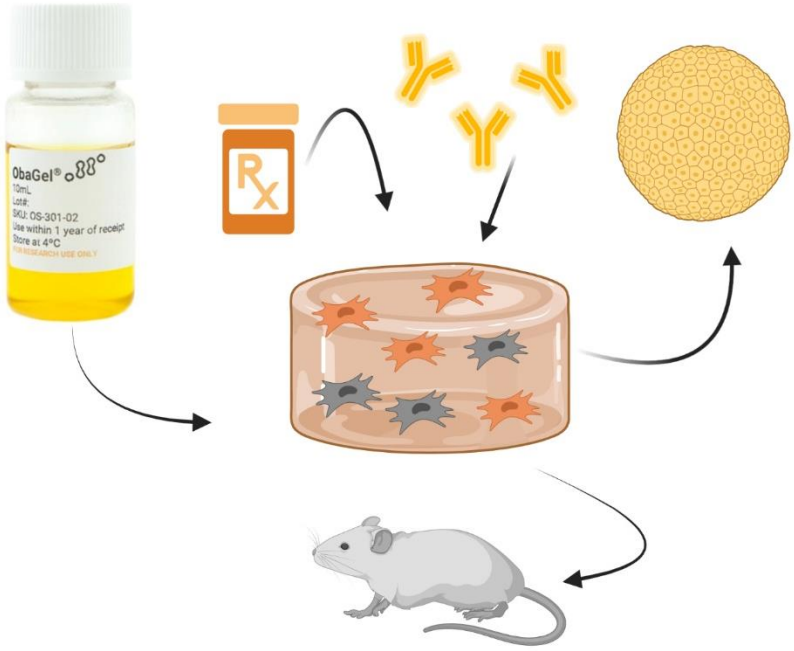
ObaGel® Culture

Catalog #: OS-313-00, OS-313-01, and OS-313-02



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Protocol
Ver 1 April 2023

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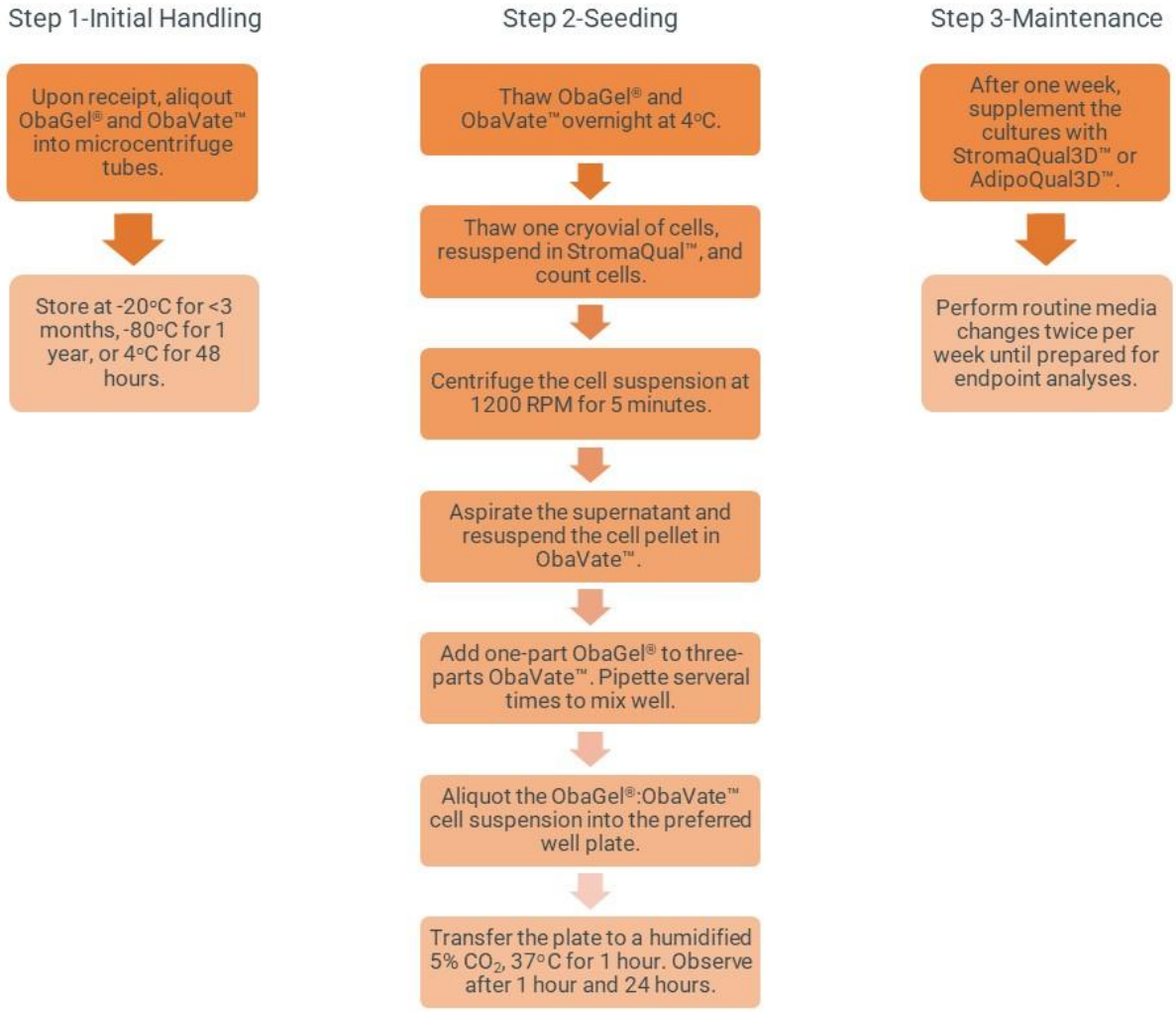

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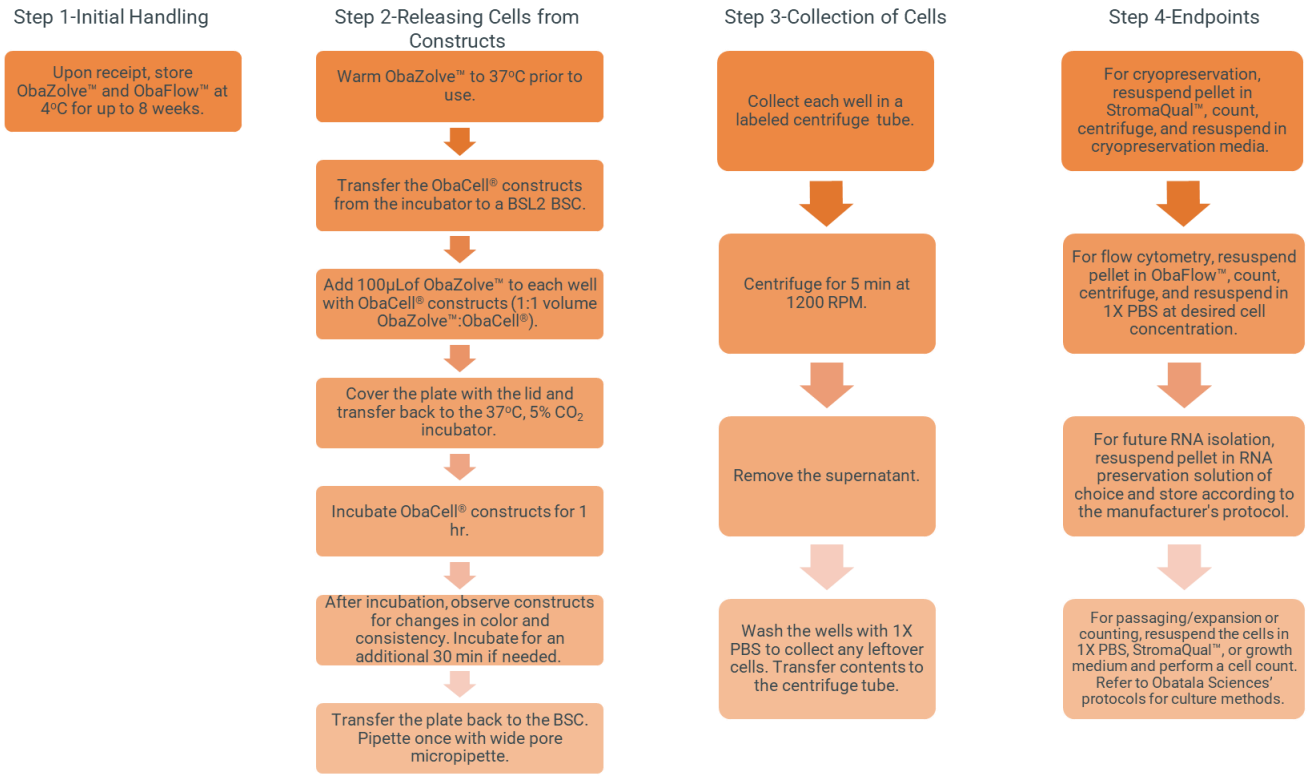

General Information

- Please refer to the safety data sheets provided with the required materials associated with ObaGel® culture for precautionary information.
- For typical appearance of and typical data obtained from cultures created using ObaGel®, please refer to our *ObaGel® Data Sheet* using the following link:
- For a brief overview of the experimental procedure, please refer to the *ObaGel® Quick Guide* using the following link:

Supplied Materials

Catalog No	Product Name	Product Description	Storage Conditions
OS-301	ObaGel®	Human-derived, 2D and 3D applications	<48 h at 4°C, >3 months at -20°C, 1 yr at -80°C
OS-302	ObaVate™	ObaGel® activating agent	-20°C until prepared to use

Recommended Materials (Not included in the kit)

Catalog No	Product Name	Product Description	Storage Conditions
OS-001	StromaQual™	Complete stromal pre-differentiation medium	4°C for 8 weeks
OS-008	Cryopreservation Medium	Freezing solution for human ASCs	4°C for 3 weeks
OS-304	ObaFlow™	Sample preparation solution for flow cytometry	4°C for 8 weeks
OS-011	Trypan Blue Exclusion Stain	Nuclear exclusion stain	4°C for 1 year
	Phosphate Buffered Saline	1X, Sterile	4°C for 8 weeks
OS-303	ObaZolve™	Dissolution reagent for cell recovery	4°C for 8 weeks
OS-101	Human Adipose-Derived Stromal/Stem Cells	Primary cells isolated from human adipose tissue	Liquid Nitrogen
OS-107	Human Stromal Vascular Fraction	Primary cells isolated from human adipose tissue	Liquid Nitrogen
OS-310	AdipoQual3D™	Adipogenic differentiation medium	4°C for 30 days after receipt
OS-309	StromaQual3D™	Pre-differentiation maintenance media	4°C for 30 days after receipt
N/A	Well plate	Sterile, tissue culture plastic	
N/A	Tubes	Sterile 50 mL, 1.5 mL, 2 mL	
N/A	Micropipettes and tips	1000, 200, 100, and 10 µL	
N/A	Wet ice	For prolonged handling	

ObaGel® Culture Setup

1. Thaw ObaGel® and ObaVate™ overnight at 4°C until they are completely thawed. Do not warm to 37°C. Keep reagents on wet ice while in use.
2. Warm growth medium of choice to 37°C.
3. Prepare a 50 mL conical tube with 4 mL of growth medium per vial of cells.
4. Thaw the cryovial of cells until the moment the ice crystals disappear from the vial.
5. Transfer the cryovial to a BSL2 BSC and add the contents of the cryovial dropwise to the 4 mL of growth medium in the conical tube.
6. Homogenize the cell suspension by pipetting several times.
7. Perform a cell count according to your laboratory's standard operating procedures.
8. Centrifuge the conical tube at 1200 RPM for 5 minutes at room temperature.
9. Aspirate the supernatant from the cell pellet and resuspend the cells in ObaVate™ at 1 million cells per mL.
10. From the 1 million cells per mL solution, calculate the total number of cells needed to create the seeding solution.
11. Calculate the volume of concentrated cell solution corresponding to the total cell number from above.
12. Subtract the volume of concentrated cell solution from the total volume of ObaVate™ needed for the seeding solution.[1]
13. Transfer the volume of concentrated cell solution to a new 50 mL conical tube.
14. Add the remaining volume of ObaVate™ needed for the seeding solution.
15. Pipette the cell solution multiple times to mix well.
16. Using a micropipette, vigorously pipette the ObaGel® prior to use.
17. Add the total volume of ObaGel® needed for the seeding solution to the 50 mL conical tube containing the ObaVate™ cell solution such that the seeding solution contains one-part ObaGel® to three-parts ObaVate™ (1:3 ratio). Pipette several times with a p1000 pipette to mix well.
18. Once the components are added, quickly transfer the necessary seeding volume of cell suspension onto the tissue culture plate growth surface. Pipette the solution between replicates to maintain homogeneous suspension.[2] [3]
19. After aliquoting, transfer the plate to a humidified 5% CO_2 incubator at 37°C. Observe after 1 hour for initial signs of gelation, and again after 24 hours to visualize cell growth.

[1] Obatala recommends accounting for pipetting error by increasing the volume by 10%.
[2] Keep suspension on ice while in use.
[3] See Appendix C for recommended seeding volumes.

ObaGel® Culture Maintenance

1. Place StromaQual3D™ and AdipoQual3D™ on a benchtop to equilibrate to room temperature.
2. Examine the ObaGel® culture under a brightfield microscope for confluency, network formation, and stability.
3. Transfer the plate and reagents to a BSL2 BSC.
4. Add the recommended volumes of media to the ObaGel® culture (refer to Appendix C). It is critical to add the media slowly and to the corner of each well. Adding media directly to the constructs could compromise their integrity.
5. Return the plate to a humidified 5% CO_2, 37°C incubator.
6. For subsequent media changes, remove the total volume of the spent media from each of the wells by tilting the plate and removing from the corner of the well.
7. Add recommended volume of fresh StromaQual3D™ or AdipoQual3D™ to their designated wells. It is critical to add the media slowly and to the corner of each well. Adding media directly to the constructs could compromise their integrity.
8. Steps (7) and (8) will occur 2x per week for 2 weeks. We recommended extending the culture time to 3 weeks of differentiation if you are executing metabolic assays such as lipolysis and glucose uptake to ensure cultures attain a homogeneous adipocyte population.

ObaGel® Endpoint Assays

1. Place ObaZolve™ in a water bath or equivalent piece of equipment to warm to 37°C prior to use.
2. Transfer the plate and reagents to a BSL2 BSC.
3. Carefully remove the spent media by tilting the plate and removing 50-60% of the media from each well.
4. Add approximately an equal volume of ObaZolve™ to each well of the plate for a 1:1 ratio v/v of ObaZolve™ solution to the construct volume.
5. Return the plate to a humidified 5% CO_2, 37°C incubator and incubate for 1 hour.
6. Transfer the plate back to the BSL2 BSC and pipette each well to observe if the gel is dissolved. If resistance is observed when pipetting, incubate the plate for an additional 30 minutes.
7. Collect each well in a tube.[4]
8. Centrifuge each tube at 1200 RPM for 5 minutes.
9. Remove the supernatant with a pipette while also ensuring that the cell pellet remains intact.
10. After resuspension of the cell pellet, proceed the following endpoints: cell passage and expansion, cryopreservation of the cells, flow cytometry setup, or preservation and storage of the cell pellet for RNA isolation.

[4] If any gel fragments are observed at the base of the well, wash each well with an additional volume of 1X PBS to collect the remaining cells. Transfer the wash solution to the microcentrifuge or conical tubes.

Appendix A: ObaGel® Culture 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
	Improper extended storage	Store at –20°C for up to one year from receipt date. Thaw and store at 4°C for no more than 48 hr prior to use.
Gelation observed during pipetting or handling	ObaGel® pre-warmed at 37°C or equivalent prior to use	Do not heat ObaGel® prior to use, maintain on ice during use
	ObaGel® not maintained at 4°C or on ice prior to use	Ensure ObaGel® is kept at 4°C or on wet ice prior to use
Contraction of 3D constructs	Physical disruption of the gel	Careful handling of the plates and manipulation of the constructs. Slowly remove and add media from edge of the well, tilt the plate to reduce shear stress at the gel-liquid interface.
	Inhomogeneous seeding solution	Pipette ObaGel®/ObaVate™ cell suspension several times immediately prior to seeding until solution appears homogenous. Pipette solution while seeding, every 3-6 replicates.
Rapid spheroid or organoid formation in 3D constructs	Too much media present at the gel-liquid interface	Collect conditioned media between feedings. Feed with recommended volumes.
	Initial seeding density of cells is too high	Reduce seeding density. Seeding density may need to be empirically determined for each cell type and lot and may depend on several factors, such as proliferation rate.
	Inhomogeneous seeding solution	Pipette several times to mix well and pipette several times between replicates

Constructs do not appear intact, or appear fragmented in wash solution	Constructs disrupted during pipetting	Pipette slowly and carefully when adding and removing reagents. Tilt the plate to collect solution from the gel-liquid interface. Use a wider-bore pipette tip to reduce shar stress on the constructs.
Gel does not appear dissolved following incubation	Incubation time not long enough	Incubate for a longer period of time, up to 30 minutes for extended cultures
	ObaZolve™ cell recovery solution not pre-warmed to 37°C prior to use	Warm ObaZolve™ to 37°C prior to use
	ObaZolve™ cell recovery solution improperly stored/handled	Store ObaZolve™ at 4°C and use within 8 weeks of receipt. Purchase fresh reagents once expired.
No visible cell pellet	Cell pellet may have been disrupted during wash steps	Decant supernatant by inverting microtube, on final wash use a micropipette to remove any remaining volume

Please contact Obatala Sciences technical support at support@obatalasciences.com if you have additional questions or concerns.

Appendix B: General Guidelines and Tips for Success

General Guidelines

1. All personnel should be trained and certified by the Principal Investigator regarding Universal Precautions and Handling of Bloodborne Pathogens.
2. All personnel should have access to relevant safety data sheets (SDS) pertaining to the use of materials.
3. All procedures should be conducted by investigators using the appropriate personal protective equipment.
4. Any waste materials should be decontaminated and disposed of using appropriate biohazard waste containers according to laboratory standard operating procedures.
5. Wear protective eyewear during handling of cryopreserved vials of cells.
6. Conduct all procedures described in the ObaGel® culture protocol in a BSL2 Biosafety Cabinet.

Tips for Success

1. Do not heat ObaGel® or ObaVate™ in a water bath or equivalent piece of equipment. Thaw both products overnight at 4°C.

2. Do not heat StromalQual3D™ or AdipoQual3D™ in a water bath or equivalent piece of equipment. Both products should be placed on a benchtop until they reach room temperature.
3. Do not centrifuge or vortex ObaGel® to remove precipitant. The precipitant is protein and will disperse upon pipetting.
4. You do not need to add culture media after initially establishing the ObaGel® culture. The ObaGel®: ObaVate™ mixture will support the cultures for one-week with no additional media.
5. When performing media changes, remove media by tilting the plate and removing media from the corner of the well. No more than 60% of spent media should be removed at a time. We suggest only removing 50% of spent media per media change.
6. The following calculations are based on the seeding of 60 wells of a 96-well plate at 5,000 cells per well. Each well has a 100 µL volume of ObaGel®: ObaVate™ cell mixture. The cell mixture is composed of a 1:3 ObaGel®: ObaVate™ ratio.
    - **# Wells to be seeded** = 60
    - **Total mixture needed** = 6 mL
    - **ObaVate™ volume** = 4.5 mL
    - **ObaGel® volume** = 1.5 mL
    - **Cells per well** = 5,000
    - **Desired cell concentration of mixture**= 50,000 per mL
    - Add **300 µL** of 1 million per mL cell solution to **4.2 mL ObaVate™** and **1.5 mL ObaGel®**
7. When culturing in a 96-well plate, we recommend only using 60 of the 96 wells and filling the outer wells with sterile 1X PBS. The constructs in the outer 36 wells tend to contract and media evaporates at a quicker rate if not monitored closely.

Appendix C: Recommended Volumes

Reagent	6-well (5 mL)	24-well (1 mL)	96-well (100 µL)
ObaGel®	1.25 mL/well	250 µL/well	25 µL/well
ObaVate™	3.75 mL/well	750 µL/well	75 µL/well
Total Construct Volume	5 mL/well	1 mL/well	100 µL/well
hSVF cells	5,000,000 cells/well	1,000,000 cells/well	100,000 cells/well
hASC cells	500,000 cells/well	50,000-100,000 cells/well	5,000-10,000 cells/well
3D Medium Feedings	2.5 mL/well	500 µL/well	50 µL/well