ObaCell® Fat-On-A-Chip Quick Guide

Catalog # OS-305-01 and OS-306-01

For a detailed protocol, refer to the *ObaCell® Fat-On-A-Chip Kit Protocol*.

Application note: for each 96-well plate, it is recommended that only the interior 60 wells are seeded, and the peripheral wells are filled with sterile 1X PBS to mediate the effects of evaporation in long-term cultures. Reagents provided are sufficient for 96 test points. The following protocol outlines the procedure for seeding 60 wells of a 96-well plate.

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
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

Suggested Materials (Not supplied)

Catalog No	Product Name	Product Description	Storage Conditions (if applicable)
OS-001	StromaQual™	Complete stromal pre-differentiation medium	4°C for 8 weeks
OS-008	Cryopreservation Medium	Freezing of 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
N/A	96-well plate	Sterile, tissue culture treated	
N/A	Tubes	Sterile 50 mL and 1.5 mL	
N/A	Micropipettes and tips	1000, 200, 100, and 10 µL	
N/A	Wet ice	For prolonged handling	

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96-well Plate ObaCell® Culture Setup

Note: All volumes and cell counts specified are associated with seeding the interior 60 wells of a 96-well plate.

STEP 1

Thaw ObaGel and ObaVate overnight at 4°C.

STEP 2

Thaw cells and resuspend in growth medium to remove DMSO.

STEP 3

Count cells and resuspend in ObaVate at 1x10^6 cells per mL.

STEP 4

Transfer volume of cell suspension to conical of ObaVate.
For ASCs, this volume is equivalent to 300,000 cells for 5,000 cells per well.
For SVF, this volume is equivalent to 3,000,000 cells for 50,000 cells per well.

STEP 5

Add the appropriate volume of ObaGel to the ObaVate cell suspension such that the solution is 1 part ObaGel and 3 parts ObaVate (1:3 ratio).
Total volume of suspension= 6 mL.

STEP 6

Pipette the solution until it is homogenous with a p1000 pipette.

STEP 7

Pipette the solution with p200 pipette into a 96-well tissue culture plate, mixing the solution between replicates.
100 µL of solution will be added to the inner 60 wells.

STEP 8

Add 100 µL of sterile 1X PBS to the outer wells of the 96-well plate.

STEP 9

Transfer the plate to a humidified 5% CO_2 incubator at 37°C.

96-well plate ObaCell® Culture Maintenance

STEP 1

Transfer StromaQual3D and AdipoQual3D to benchtop to equilibrate to room temperature.

STEP 2

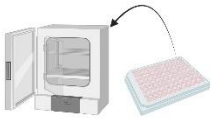
Examine the cultures for confluency, network formation, and stability.

STEP 3

Add recommended volume of StromaQual3D or AdipoQual3D to each well.
Media should be added slowly to the corner of each well.

STEP 4

Transfer the plate to a humidified 5% CO_2 incubator at 37°C.



STEP 5

Media changes will occur 2x per week for 2 weeks.
~30-40 µL media will be left in each well between feedings.
Remove 30 µL of media prior to adding fresh media by tilting the plate and removing 30 µL at the corner of the well, ensuring the constructs are not disturbed.
Total volume of AdipoQual3D required: 17.1 mL
Total volume of StromaQual3D required: 1 mL

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96-well Plate ObaCell® Harvest

STEP 1

Warm ObaZolve to 37°C prior to use.

STEP 2

Carefully remove the media from all 60 wells by tilting the plate and removing from the corner of each well.
~30-40 µL media will be removed from each well.

STEP 3

Add an equal volume of pre-warmed ObaZolve to each well for a 1:1 v/v of ObaZolve solution to construct volume.

STEP 4

Transfer the plate to a humidified 5% CO_2 incubator at 37°C for 1 hour.

STEP 5

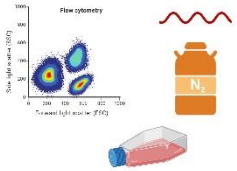
Pipette each well to observe if gel is dissolved.
If resistance is observed, incubate the plate for an additional 30 minutes.

STEP 6

Collect each well in a conical or microcentrifuge tube. Centrifuge each tube at 1200 RPM for 5 minutes.
If any gel fragments remain at the base of the wells, wash each well with 1X PBS to collect remaining cells.

STEP 7

Remove the supernatant. After resuspension of the cell pellet, proceed with the following endpoints: cell passage and expansion, cryopreservation of cells, setup for flow cytometry, or preservation and storage of cells for RNA isolation.



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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. Store on wet ice while in use.
2. Do not heat StromalQual3D™ or AdipoQual3D™ in a water bath or equivalent piece of equipment. Equilibrate to room temperature prior to use.
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 changes.
5. When performing media changes, remove media by tilting the plate and removing media from the corner of the well. Take care as to not disturb the constructs while removing conditioned medium. Remove the total volume of conditioned media present prior to each 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® for a final cell suspension at 50,000 cells/mL**
7. When culturing in a 96-well plate, we recommend seeding 60 interior wells and filling the peripheral wells with sterile 1X PBS to mediate evaporation of long-term cultures.

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