Obatala Sciences' Protocol 302
How Do I Recover Cells from ObaGel® with ObaZolve™?

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Reagents, Materials, and Equipment

- Obatala Sciences' ObaZolve™ (Catalog #OS-303)
- Obatala Sciences' ObaFlow™ (Catalog #OS-304)
- Phosphate buffered saline (1X) or equivalent product
- Benchtop centrifuge
- Conical centrifuge tube
- BSL2 Biological Safety Cabinet
- 37°C, 5% CO_2 incubator
- Micropipette and pipette tips
- Microcentrifuge tubes

Protocol

Initial Handling of Your ObaZolve™ and ObaFlow™

1. ObaZolve™ and ObaFlow™ will be shipped on blue ice. Upon receipt, store at 4°C for up to 8 weeks.

Releasing Cells from ObaGel™ with ObaZolve™

1. Warm ObaZolve™ to 37°C prior to use.
2. Transfer the ObaCell® constructs from the 37°C incubator to a biological safety cabinet (BSL2).
3. Transfer ObaZolve™ to the BSL2 biological safety cabinet. Wipe down the exterior of the bottle with 70% ethanol.
4. Add an approximately equal volume of ObaZolve™ to each well containing the ObaCell® constructs for a 1:1 ratio v/v of ObaZolve™ solution to the ObaCell® culture volume.
    a. For a 96-well culture, add 100 µL of ObaZolve™ to each well.
    b. For a 24-well culture, add 1 mL of ObaZolve™ to each well.
    c. For a 6-well culture, add 5 mL ObaZolve™ to each well.
5. Cover the plate with the lid, transfer to a 37°C CO_2 incubator, and incubate for 1 hr.
6. After 1 hr incubation, observe the ObaCell® constructs with a brightfield microscope to observe noticeable changes in the constructs including gel

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retraction and cell aggregation at the well's center. If no noticeable changes are observed, incubate the constructs for an additional 30 min.
(Note: These values are optimized for the density of the cultures. For cultures that appear especially dense, either due to cell proliferation or initial seeding density, incubate for an additional 30 minutes.)

7. Transfer the plate to the BSL2 biological safety cabinet. Using a micropipette equipped with wide bore sterile pipette tips, pipette the contents of each well once. Then, transfer the total well contents to a microcentrifuge tube. If any gel fragments remain at the base of the well, wash each well with an additional volume of 1X PBS to collect remaining cells. Transfer the wash solution to the microcentrifuge tube.
8. Centrifuge the cell suspension for 5 minutes at 300x g (1200 rpm) to retrieve a cell pellet.
9. Remove the supernatant from the cell pellet with a micropipette. Do not aspirate and risk disrupting any remaining gel fragments near the pellet.
10. Proceed with the endpoints outlined below.

3D ObaGel® Culture Endpoints

a. Recovery of cells for passaging/expansion or counting
    i. Resuspend cells in 1X PBS, StromaQual™ Stromal Medium, or growth medium of choice. Perform a cell count to quantify the total number of live cells and seed in 2D or 3D at the desired seeding density.
    ii. Refer to Obatala Sciences' protocols 301 and 101 for 3D and 2D culture methods, respectively.
b. Recovery of cells for cryopreservation
    i. Resuspend cells in StromaQual™ Stromal Medium or growth medium of choice. Perform a cell count to quantify the total number of live cells. Centrifuge to retrieve a cell pellet and resuspend in cryopreservation medium at the desired cell concentration.
    ii. Refer to Obatala Sciences' protocol 103 for cryopreservation methods.
c. Recovery of cells for flow cytometry using ObaFlow™
    i. Resuspend the cell pellet in ObaFlow™. Perform a cell count to quantify the total number of live cells. Centrifuge to retrieve a cell pellet. Proceed with routine sample preparation protocol for flow cytometry.
    ii. Example: resuspend the cell pellet in 270 µL of PBS. Distribute 50 µL aliquots each to five Eppendorf tubes. Add pre-determined volume of antibody of interest to each cell aliquot. Incubate the contents in the dark for 60 min at room temperature. Add 1 mL of 1X PBS or wash buffer of choice per tube. Centrifuge at 300x g for 5 minutes at

room temperature. Decant/aspirate supernatant. Repeat wash steps a total of 3 times. Resuspend the pellet in 400 µL of 1X PBS and transfer directly to a flow cytometry capped tube.

iii. *Optional: At this point, cells may be fixed in 4% formaldehyde for 30 minutes and then stored at 4°C until ready to run flow cytometry evaluation of labeling.*

d. Preservation and Storage of RNA

i. Resuspend the cell pellet in RNA preservation solution of choice. Proceed with sample storage or RNA isolation as outlined in the manufacturer's protocol.

Recommended Protocols

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®?
Obatala Sciences' Protocol 401–How Do I Create 2D 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!

Appendix A: Cell Recovery from ObaGel® with ObaZolve™ Workflow

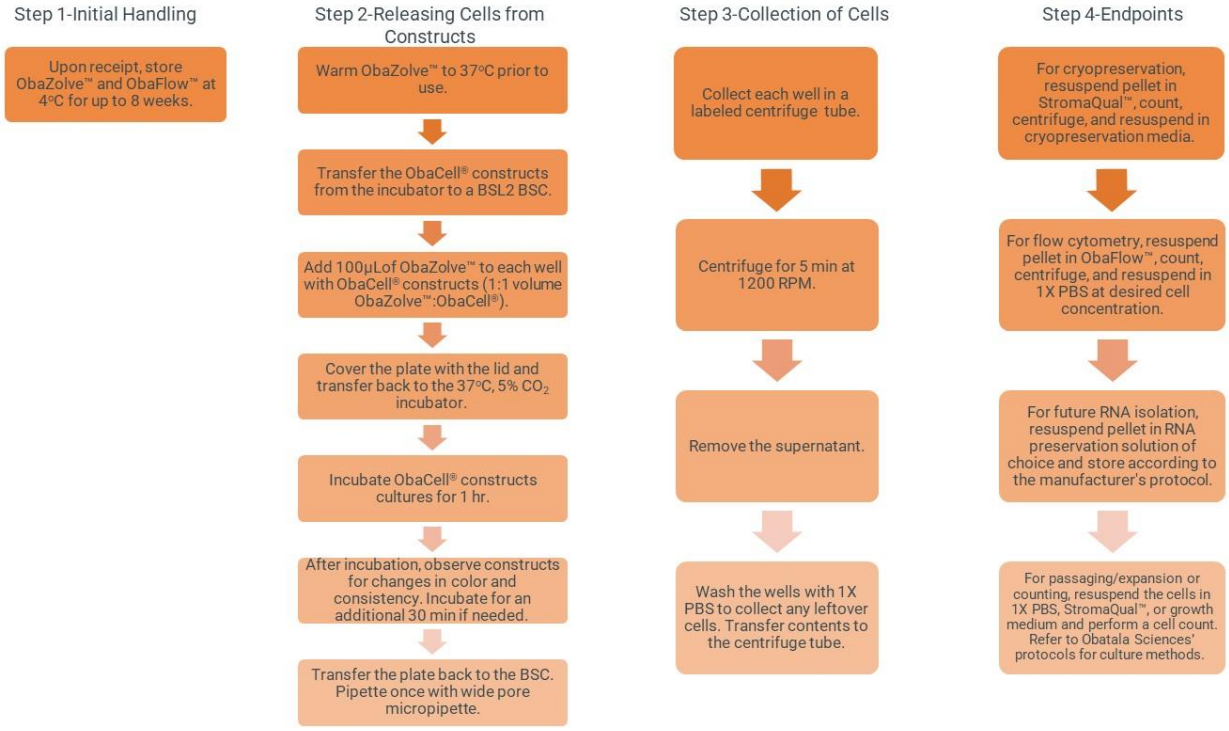

Figure 1. Workflow diagram outlining the steps required to recover cells from 3D ObaGel® cultures. Steps for cell recovery with ObaZolve™ include: initial handling of ObaZolve™, releasing the cells from ObaCell® cultures, collection of cells, and procedures for follow-on studies with cells recovered from ObaCell® cultures.

Appendix B: Troubleshooting

Problem	Reason	Solution
Gel does not appear dissolved following incubation	Incubation time not long enough	Incubate for a longer period of time, up to 1.5 hrs 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.
RNA yield – reduced quality and quantity	Not using ice-cold 1X PBS following cell recovery	Use ice-cold 1X PBS for wash steps
	Not devoting enough replicates per condition	Devote 3-6 replicate wells per experimental condition to yield enough quality RNA for endpoints