Obatala Sciences™ Protocol 303
How Do I Recover Cells from ObaGel® with ObaZolve™?

Written by: Obatala Sciences™ Staff
Last Updated: July 2021

Reagents, Materials, and Equipment

- Obatala Sciences' ObaZolve™ (Catalog #OS-303)
- Obatala Sciences ObaFlow™ (Catalog #OS-304)
- Conical centrifuge tube

Protocol

Initial Handling of Your ObaZolve™ and ObaFlow™

1. When you receive the package containing your ObaZolve™ and ObaFlow™ on dry ice, transfer it to a 4° C refrigerator for overnight or longer until fully thawed. Then, aliquot the contents from each container into separate appropriately labeled tubes containing the desired smaller volumes for each and transfer them separately to a -80°C freezer for storage periods of > 3 months (shelf life, up to 1 year). If you plan to use the products within < 3 months, they can be safely stored at -20°C. You may store ObaFlow™ and in its original container and subject to multiple freeze/thaw cycles without loss of activity; it can be stored at -20°C.

Releasing Cells from ObaGel™ with ObaZolve™

1. If needed, thaw ObaZolve™ and ObaFlow™ overnight by transferring from -20°C or -80°C freezer to a 4°C refrigerator. On the day of seeding/usage, warm ObaZolve™ to 37°C immediately prior to use.
   (Note: You may have received your products already thawed. Products can be stored at 4°C for >1 month, but should be transferred to -20°C for prolonged storage. Do not place the ObaZolve™ at room temperature or 37°C for extended periods of time since this will substantially shorten the shelf-life of the product.)
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.

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

5. Cover the plate with the lid and transfer to a 37° C CO_2 incubator. For ObaCell® constructs cultured for <7 days, incubate for 15 minutes; for constructs cultured for >7 days, incubate for 30 minutes.
   (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 30 minutes.)
6. After a 15-30 minute incubation period, the ObaCell® constructs should appear partially liquified and a colorimetric change will have occurred.
7. Transfer the plate to the BSL2 biological safety cabinet. Using a micropipette equipped with sterile pipette tips, pipette the contents of each well several times until the well contents appear homogenous, liquified, and resistance to pipetting becomes significantly reduced; approximately 5-8 times per well. Transfer the total well contents to a centrifuge tube. If any gel fragments remain at the base of the well, wash each well with ObaZolve™ to collect remaining cells.
8. After all wells have been collected, further dilute the contents of each centrifuge tube with 5-10mL sterile 1X PBS, or 25% of the volume, whichever is greater. Pipette the contents several times to break up any remaining gel fragments
   (Note: This additional dilution will help to dissociate cells from any remaining gel fragments and create a homogenous suspension.)
9. Centrifuge the cell suspension for 5 minutes at 300 X g (1200 rpm) to retrieve a cell pellet.
10. Decant the supernatant from the cell pellet, do not aspirate and risk disrupting any remaining gel fragments near the pellet.
11. Resuspend the pellet in 10-20mL sterile 1X PBS and centrifuge for 5 minutes at 300 X g (1200 rpm).
12. Repeat steps 10-11 for a second wash.
13. Following the second wash, decant the supernatant from the cell pellet. The cell pellet should appear intact and no remaining gel fragments should appear at the base of the conical. Proceed with the endpoints outlined below.
    a. Recovery of cells for passaging/expansion
        i. Resuspend cells in StromaQual™ Stromal Medium or 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™ protocol 301 and 302 for 3D and 2D culture methods, respectively.
    b. Recovery of cells for cryopreservation
        i. Resuspend cells in StromaQual™ Stromal Medium or 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 minutes at room temperature. Add 1ml of PBS or wash buffer of choice per tube. Centrifuge at 300 X g for 5 min at room temperature. Decant/aspirate supernatant. Repeat wash steps a total of 3 times. Resuspend the pellet in 400 µl of PBS and transfer directly to a flow cytometry capped tube. *Optional: At this point, cells may be fixed in 4% formaldehyde for 30 minutes and then stored at 4ºC 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 302 – 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 Science 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!