Obatala Sciences' Protocol 303
How Do I Recover 3D ObaCell® Cultures?

Written by: Obatala Sciences' Scientific Team
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Reagents, Materials, and Equipment

- Live 3D ObaCell® Cultures (Catalog #OS-312)
  - Supported formats: 96-well plate, 384-well plate
- Obatala Sciences' ObaVate™ (Catalog #OS-302)
- 3D Culture Medium
  - Obatala Sciences' StromaQual3D™ (Catalog #OS-309)
  - Obatala Sciences' AdipoQual3D™ (Catalog #OS-310)
- 70% ethanol
- BSL2 Biological Safety Cabinet
- 37°C, 5% CO_2 incubator
- Sterile paper towel or kimwipe
- Microplate plate seal
- Micropipette and pipette tips
- Parafilm
- Wet ice for prolonged handling

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 live 3D ObaCell® Cultures (Catalog #OS-312), carefully unpack the shipment so that the plate(s) remain upright with minimal physical disruption. The wells should appear to be filled with media with a dense region of cells near the base of the well where the 3D constructs are located.
   a. Transfer plate(s) to a BSL2 biological safety cabinet. Remove the plate lid and inspect the plate seal for evidence of leakage.

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b. With plate seal still intact, image several wells per condition with a brightfield microscope. Take note of any regions within wells that show evidence of network formation or disruption.
c. Return the plate(s) to a BSL2 biological safety cabinet. Carefully remove the plate seal and cover the plate with the plate lid. Transfer the plates to a humidified 5% CO_2, 37°C incubator.

2. A representative from the Obatala Sciences' team will be in communication regarding the status of the plate(s) post-arrival. We request that you share any photomicrographed images of the plate to verify the integrity of the constructs.
    a. We ask that you continue to observe the plates for 48 hours post-arrival.

Re-acclimation of 3D ObaCell® Cultures

1. Once the integrity of the plates has been verified and no evidence of contamination is observed after 48 hours, return plate(s) to a humidified 5% CO_2, 37°C incubator for up to 3 additional days to complete the acclimation period.
   (Note: This acclimation period is critical for the recovery of cell networks. We recommend allotting a period of 5 days post-arrival before further manipulating the cultures.)
2. After the acclimation period, return the plate(s) to a BSL2 biological safety cabinet. 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 disrupt the constructs.
    a. Remove volume slowly and with consistent speed as to not draw up the gel from below the liquid interface.
    b. The constructs will appear as a dense region of gel at the base of the well and should appear distinct from the liquid interface when tilting.
    c. Proceed with feedings or alternate endpoints. Do not allow the 3D constructs to dry out after removing conditioned medium.
3. Replace conditioned media with StromaQual3D™ for maintenance of 3D cultures or AdipoQual3D™ for adipogenic differentiation of cultures.
    a. We recommend pipetting the new volume of medium against the wall of the well to reduce any shear stress to the constructs when the liquid flows onto the gel interface.
    b. Feed constructs with 25-50 µL of 3D media per well for 96-well plate format.

Recommended Protocols

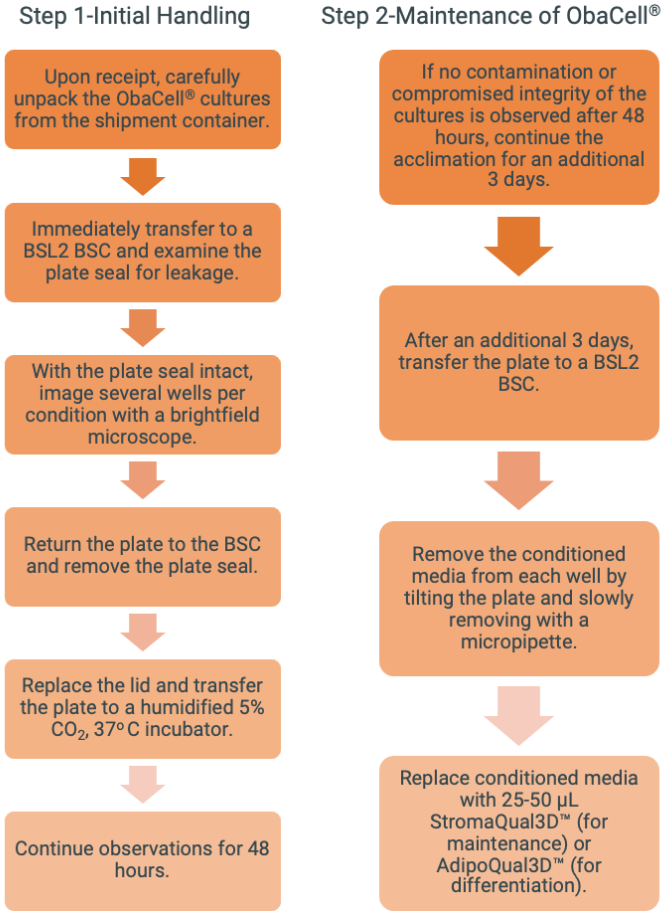
Obatala Sciences' Protocol 302–How to Recover Cells from ObaGel® with ObaZolve™?
Obatala Sciences' Protocol 304–How to Fix 3D ObaCell® Cultures?

After you have recovered 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!

Appendix A: Handling and Maintenance of ObaCell® Cultures Workflow



Appendix B: Troubleshooting

Problem	Reason	Solution
Constructs do not appear intact following shipping and recovery	Constructs may have been improperly handled during shipping	Contact a representative at Obatala Sciences, 504-300-0266