



## Obatala Sciences' Protocol 304 How Do I Fix 3D ObaCell® Cultures?

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Last Updated: January 2024

### Reagents, Materials, and Equipment

- ◆ Live 3D ObaCell® Cultures (Catalog #OS-312)
  - Supported formats: 24-well plate, 96-well plate, 384-well plate
- ◆ 10% neutral buffered formalin
- ◆ Phosphate buffered saline (1X) or equivalent product
- ◆ Fluorescent dye
  - Neutral lipid stain: BODIPY 493/503
  - Nuclear stain: Hoechst 33342 460/490
  - Mitochondrial stain: MitoTracker Red 581/644
- ◆ Parafilm
- ◆ Aluminum foil
- ◆ Conical tubes
- ◆ Micropipette and tips

### 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

#### Reagent Preparation

1. Prepare working solutions for each fluorescent dye according to manufacturer's protocol.
  - a. Perform necessary dilutions in 1X PBS. Alternatively use a phosphate-free buffer.
2. Prepare 10% neutral buffered formalin.

## Fixation and Staining of ObaCell® Cultures (Catalog #OS-312)

1. Transfer plate to a BSL2 biological safety cabinet to collect conditioned or spent media from each well. All other handling may be done on the benchtop.
  - a. Tilt the plate to allow liquid to collect at the base of the well. Use a micropipette to aspirate the volume slowly with consistent speed as to not disrupt the constructs.
  - 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.
2. Add 10% formalin to each well by pipetting slowly against the wall of the well to allow the liquid to flow across the surface of the constructs.
  - a. We recommend using 500  $\mu$ L and 50  $\mu$ L formalin for a 24-well plate and 96-well plate respectively. This volume will adequately submerge the constructs.
3. Seal the plate with parafilm and incubate at 4°C for 24 hours.
4. Following fixation, wash the constructs 3x with 1X PBS or phosphate-free buffer of choice using the method detailed above to reduce shear stress on the constructs.
5. Add working solution of primary fluorescent dye to each well. Use volumes as recommended by manufacturer's protocol.
6. Seal the plate with parafilm, cover the plate in foil to minimize light exposure, and incubate at 4°C overnight.

*(Note: Overnight incubation is more than sufficient for most stains and can be reduced with experimentation. As a starting point, we recommend at least doubling the recommended incubation time from manufacturer's protocols for 2D cultures).*
7. Following incubation, wash the constructs 3x with 1X PBS or phosphate-free buffer of choice using the method detailed above to reduce shear stress on the constructs.
8. Proceed with counterstaining or store constructs in 1X PBS at 4°C light-blocked with foil prior to imaging.

*(Note: Constructs should be stable at 4°C stored in 1X PBS for 2-3 weeks. It is not recommended that plates be stored for >6 weeks.)*

## Recommended Protocols

Obatala Sciences' Protocol 303–How to Recover 3D ObaCell® Cultures?

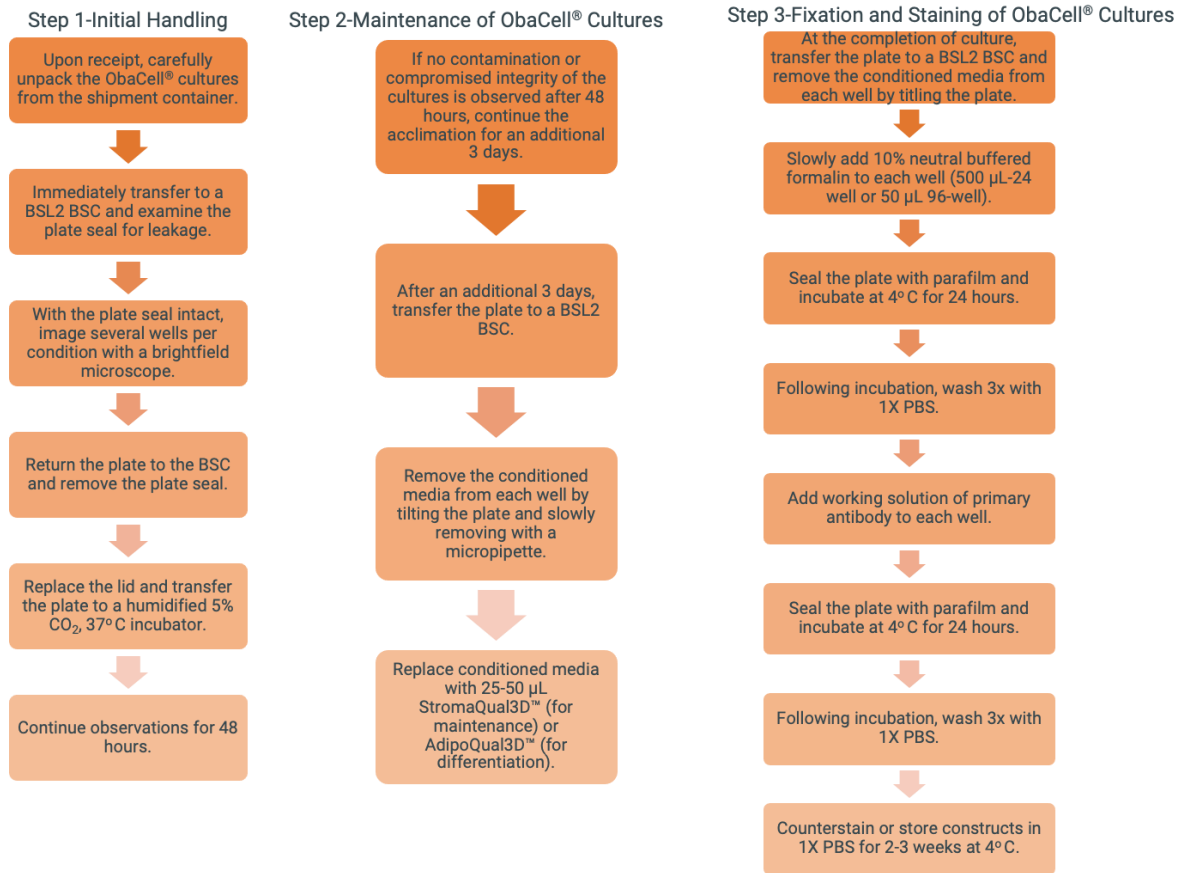
Obatala Sciences' Protocol 301-How Do I Create 3D Cultures with ObaGel®?

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: ObaCell® Culture Fixation and Staining Workflow



**Figure 1. Workflow diagram outlining the steps required to fix and stain ObaCell® cultures.** Steps for protocol 304 include: initial handling ObaCell® cultures, maintenance of the established ObaCell® cultures, fixation of ObaCell® cultures, staining of ObaCell® cultures, and storage of stained ObaCell® cultures.

## Appendix B: Troubleshooting

<b>Problem</b>	<b>Reason</b>	<b>Solution</b>
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 shear stress on the constructs.
Increased background staining	Wash solution not fully penetrating the constructs	Repeat wash steps and consider incubating the constructs with the wash solution prior to removal. Note when the wash solution remains clear after incubation.