Obatala Sciences™ Protocol 305
How Do I Fix 3D ObaCell® Cultures?

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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
- 1x PBS
- Fluorescent dye
  - Neutral lipid stain: BODIPY 493/503
  - Nuclear stain: Hoechst 33342 460/490
  - Mitochondrial stain: MitoTracker Red 581/644
- Parafilm

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.

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    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 500uL and 50uL 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 4º 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 4º 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 4ºC light-blocked with foil prior to imaging.
   (Note: Constructs should be stable at 4ºC *stored in 1X PBS for 2-3 weeks. It is not recommended that plates be stored for >6 weeks.)*

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.

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