Obatala Sciences' Protocol 403
How Do I Use Obatala Sciences' ObaGel®-ECM to Grow Cells in 2D?

Written by: Obatala Sciences' Scientific Team
Last Updated: January 2024

Reagents, Materials, and Equipment

- Obatala Sciences' Human Adipose-Derived Stromal/Stem Cells (Catalog# OS-101), Human Stromal Vascular Fraction Cells (Catalog# OS-107-01), or equivalent cryopreserved primary cell product
- 70% ethanol
- Sterile paper towel or kimwipe
- Conical tubes
- Multi-well plate, or equivalent plasticware suitable for cell culture
- Obatala Sciences' ObaGel®-ECM^{A} (Catalog# OS-314)
- Obatala Sciences' ObaGel®-ECM^{B} (Catalog# OS-314)
- Cell culture medium of choice
- Phosphate buffered saline (1X) or equivalent product
- P-1000 pipette and tips
- 37°C, 5% CO_2 incubator
- Serological pipet
- Wet ice for prolonged handling

Calculations

Plate Size	Surface area per well (cm^2)	Coating Volume* (mL)	Media Volume-Total (mL)	Media Volume-Feedings (mL)
24-well	1.9	0.25	0.5	0.25
48-well	0.95	0.125	0.3	0.125

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

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 always using appropriate personal protective equipment. Any waste materials should be decontaminated (bleached) and disposed of using appropriate biohazard waste containers.

Protocol

Coating of Culture Vessels

Note: Perform all tasks in Level 2 Biosafety Cabinet using aseptic technique.

1. Mix ObaGel®-ECM^{A} and ObaGel®-ECM^{B} at a 1:1 (v/v) ratio. Pipette slowly to avoid creating bubbles in the mixture. Use a serological pipette to thoroughly mix the hydrogels.
2. Add appropriate volume of coating solution to each well and swirl the plate to evenly coat the surface of the well.
    a. See *Calculations* for recommended coating volume.
3. Transfer plate to cell culture incubator at 37°C for 30 minutes, or until crosslinking has occurred.
4. Seed cells in an additional volume of cell culture media at the desired concentration, experimentally determined based on the surface area and cell type used.
    a. See *Calculations* for recommended media volume.
5. For maintenance after cell seeding, change media every 2-3 days by removing half of the well volume and replacing it with an equal volume of media.
    a. See *Calculations* for recommended media volume-feedings.
    b. Always add media to side wall of the well, and never directly on top of the gel.

Note: If gel coating is too thick or is disrupted, it may detach from the culture surface and float within the media.

Appendix A: ObaGel®-ECM Coating Procedure Workflow

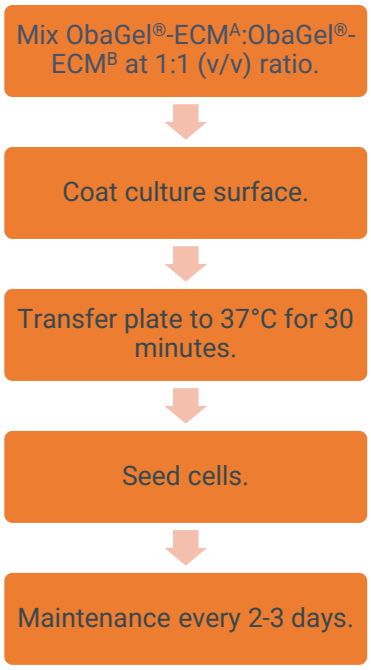

Figure 1. Workflow diagram outlining the steps required to create 2D cultures with ObaGel®-ECM. Steps for protocol 403 include: coating of culture vessels with ObaGel®-ECM and maintenance of the established ObaGel®-ECM cultures.

Appendix B: Troubleshooting

Problem	Reason	Solution
High air bubble content when initially mixing the solutions	N/A	Use 5- or 10-mL serological pipette instead of micropipette tips when mixing to reduce the air bubbles.
Observe lifting of coating	Pipetting directly onto vessel surface	Pipet media onto corner of the vessel.