Obatala Sciences' Protocol 402
How Do I Use Obatala Sciences' ObaGel®-Coating as a Protein Surface Coating?

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®-Coating (Catalog# OS-315)
- 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 - Pre-Seeding (mL)	Media Volume - Total (mL)	Media Volume – Feedings (mL)
12-well	3.8	1	0.5	1	0.5
24-well	1.9	0.5	0.25	0.5	0.25
48-well	0.95	0.125	0.125	0.3	0.125
T-75 Flask	75	3	0-5	10	5

**Volume of diluted coating solution*

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

Storage

1. Upon receipt of product, store at -20°C. Before use, thaw at 4°C overnight. Aliquot into working volumes and freeze at -20°C for future use to reduce freeze/thaw cycles.
    a. Store at -20°C for 12 months after receipt.
    b. Store at 4°C for 7 days after thawing.

Coating Plates

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

a. Dilute ObaGel®-Coating solution to desired protein concentration in 1X DPBS. (ObaGel®-Coating is provided at ~0.7-1 mg/mL protein concentration) or use the 100% concentration without diluting.
Note: Homogenize the diluted mixture using a 5 mL serological pipet to avoid creating bubbles.

2. Add appropriate volume of coating solution to each well and swirl the plate to evenly coat the surface of the well. Then, leave plate at RT overnight at 4°C wrapped in parafilm to prevent evaporation.
3. Transfer plate(s) to BSC and use a pipette to remove excess coating solution and discard.
4. Add appropriate volume of cell culture media to each well to condition the culture surface. Return to the incubator to allow surface and temperature to equilibrate to 37°C for 30 minutes.
    a. Equilibrate plate temperature to RT if stored at 4°C overnight before adding media.
    b. See *Calculations* for recommended media volume.
5. 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.
6. 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.

Note: We recommend using Accutase® Cell Detachment Solution when harvesting cells from coated surfaces for optimal cell yield.

Appendix A: ObaGel®-Coating 2D Procedure Workflow

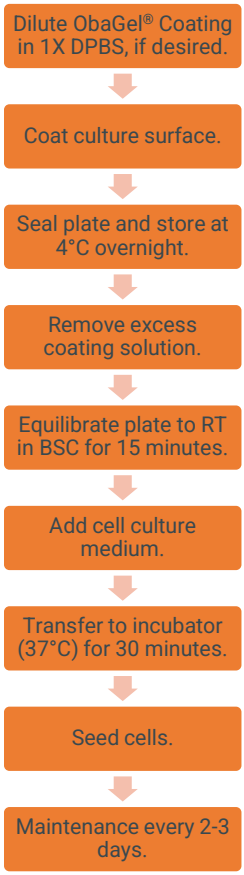

Figure 1. Workflow diagram outlining the steps required for 2D coating applications with ObaGel®-Coating. Steps for coating with ObaGel®-Coating include: Initial handling of ObaGel®-Coating, coating of culture vessel with ObaGel®-Coating, seeding of the ObaGel®-Coating cultures, and maintenance of the established ObaGel®-Coating cultures.

Appendix B: Troubleshooting

Problem	Reason	Solution
Gelation observed when using 100% ObaGel®-Coating	ObaGel®-Coating gelation will occur at RT but will experience loss of integrity at 37°C.	Allow coated vessel to incubate overnight at 4°C and not at RT.
Cells clumping or sticking to culture vessel after treating with cell detachment enzyme	ObaGel®-Coating contains collagen and promotes matrix deposition which can contribute to cell surface adherence.	We recommend using Accutase® Cell Detachment Solution for optimal cell yield.