Quick Guide

iCell® Retinal Pigment Epithelial Cells

Handling and Storage

Upon receipt, immediately transfer the cryovial to liquid nitrogen storage.

Preparing Cell Culture Surfaces

For best results, use vitronectin-coated vessels. Alternatively, tissue culture-treated vessels can be directly plated with cells in serum-containing medium.

1. Prepare 2.5 µg/ml vitronectin. For example to prepare 1 ml, combine:
   - 990 µl CellAdhere Dilution Buffer (STEMCELL Technologies, # 07183)
   - 10 µl of 250 µg/ml Vitronectin XF (STEMCELL Technologies, # 07180)
2. Coat vessel by adding the recommended coating volume per well (**Table 1**).
3. Incubate for ≥1 hour at room temperature.
4. Remove coating solution immediately before plating the cells (no rinse needed).

Preparing the Medium

Cells can be plated and cultured in either serum-free or serum-containing medium.

1. Prepare medium (**Table 2**); sterile filter using a 0.2 µm PES filter unit.
2. Store medium at 4°C for up to 2 weeks.

Thawing the Cells

1. Warm 25 ml of medium to room temperature.
2. Dispense 8 ml of medium into sterile 15 ml centrifuge tube.
3. Thaw the cryovial in a 37°C water bath for 3 minutes; clean with 70% ethanol.
4. Transfer the cells to the centrifuge tube containing the 8 ml of medium.
5. Rinse the cryovial with 1 ml of medium and transfer to centrifuge tube.
6. Centrifuge the cells at 300 x g (~1,000 rpm) for 5 minutes; discard the supernatant.

Plating the Cells

1. Check the Certificate of Analysis to obtain the number of expected cells.
2. Resuspend the cells at ~0.5 x 10^6 cells/ml.
3. Add the cells to vessel using the recommended culture volume per well (**Table 1**).
4. Incubate the cells at 37°C, 5% CO_2.

Replacing the Medium

Feed the cells every 2 days by replacing the culture volume (**Table 1**) with an aliquot warmed to room temperature.

For most applications, the cells should be cultured for a minimum of 21 - 28 days (**Figure 2**).

Note: *The cells are for LIFE SCIENCE RESEARCH USE ONLY. See www.cellulardynamics.com/product-warranty/ for USE RESTRICTIONS applicable to the cells and other terms and conditions related to the cells and their use.*

Contacting Technical Support

Email: support@cellulardynamics.com

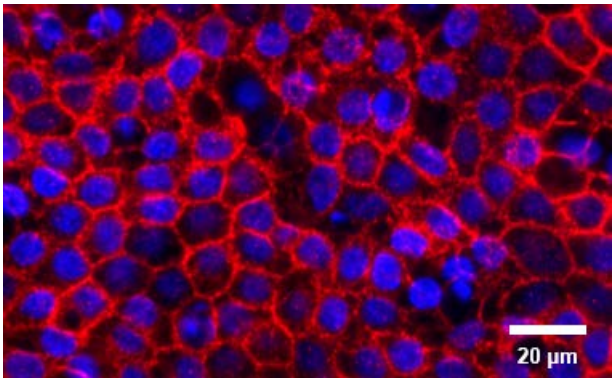

Figure 1: iCell Retinal Pigment Epithelial Cells *were cultured for 31 days in serum-free medium: mature RPE marker BEST1 (red) and nuclei (blue).*

Culture Vessel	Surface Area (cm^2)	Coating Volume (ml)	Culture Volume (ml)	Cell Number (cells)
6-well	9.5	2	3	1.5 x 10^6
12-well	3.8	0.8	1.2	0.6 x 10^6
12-well Transwell	1.12	0.24	0.35	0.175 x 10^6
24-well	1.9	0.4	0.6	0.30 x 10^6
48-well	0.95	0.2	0.3	0.15 x 10^6
96-well	0.32	0.07	0.1	0.05 x 10^6

Table 1: Cell Culture Volumes and Measures (per well)

Component	Volume (ml)	Final Concentration
MEM alpha ThermoFisher, # 12571-063	93.3	91.3%
KnockOut SR* ThermoFisher, # 10828-028	5	5%
N-2 Supplement ThermoFisher, # 17502-048	1	1%
Hydrocortisone, 50 µM Sigma, # H6909	0.11	55 nM
Taurine Sigma, # T0625 → prep 50 mg/ml**	0.5	250 µg/ml
Triiodo-L-thyronine (T_3) Sigma, # T5516 → prep 20 µg/ml** Dilute 1:1,000 immediately before use	0.07	14 pg/ml
Gentamicin, 50 mg/ml ThermoFisher, # 15750-060 → optional	0.05	25 µg/ml

Table 2: Medium Preparation (adapted from JoVE 45, e2032)

* Alternatively, 5% fetal bovine serum can be used.

** Follow manufacturer's guidelines; stock solution may be aliquoted and frozen.

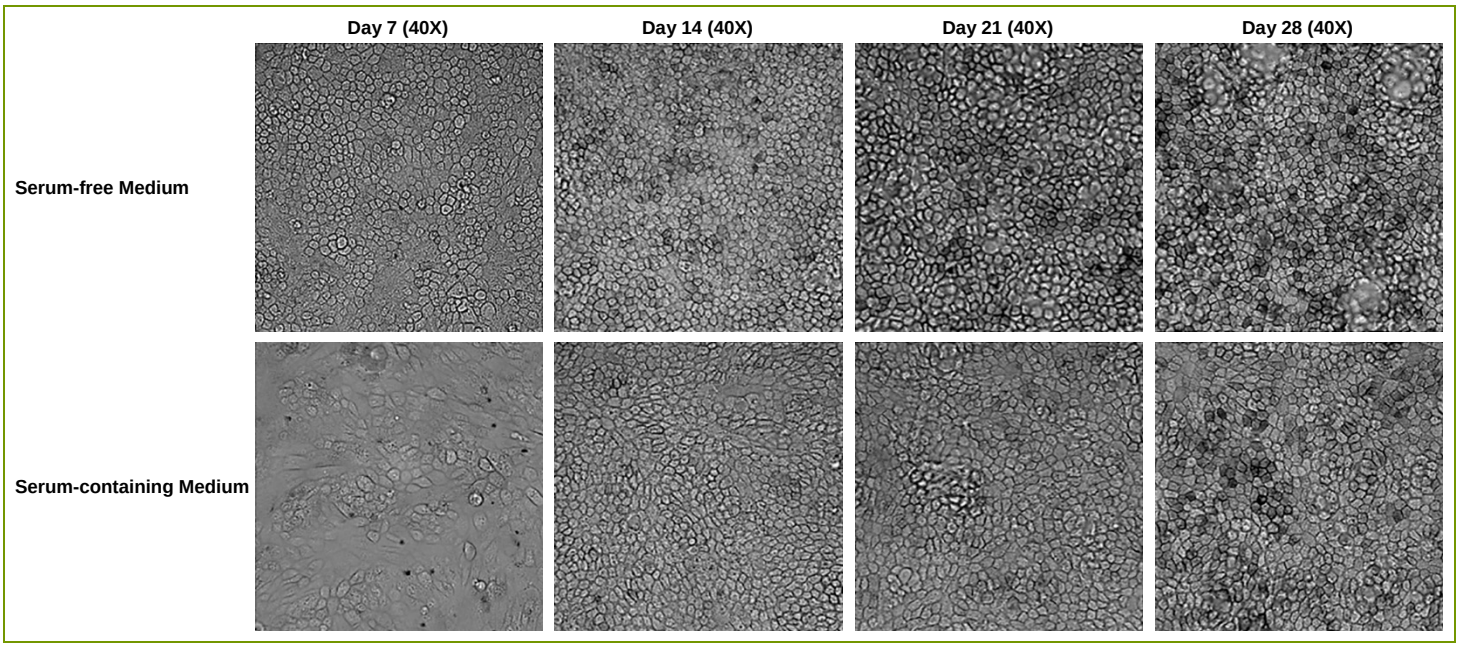

Figure 2: iCell Retinal Pigment Epithelial Cells *can be cultured under serum-free or serum-containing conditions to form a tight monolayer with polygonal cell morphology that becomes increasingly pigmented with time in culture. Images were taken with a 40X objective.*

Revision History

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