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🌐 HTMCs 3D culture method using Corning Matrigel Matrix

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Protocol status: Working

Cell Cultures

- 1 Human trabecular meshwork cells (HTMC) and their Growth Medium with FBS, the Trabecular Meshwork Growth Medium (TMGM), from Cell APPLICATION INC.

Maintain the HTMCs at 37°C in a humidified atmosphere containing 5% CO₂, according to the manufacturer's indications. Sub-cultured HTMCs by TripLE™ Express (Invitrogen Life Technologies) treatment when the original flask is approximately 75% confluent.

Cells embedded

- 2 Slightly centrifuge $2.5 \times 10^5/\text{cm}^3$ cells for 5 min at 90 rcf, discard the supernatant and gently resuspend the cell pellet in undiluted Matrigel™ at 4°C, following the application of this formula: 1d

Volume of added Matrigel (ml)/culture chamber growth area (cm²).

i.e.: $0.2 \text{ ml} / [(1.77/2)^2 3.14] = 0.113 \text{ cm} \rightarrow 1.13 \text{ mm thickness/growth area}$.

Gently transfer the embedded HTMCs by pipette into culture chambers and add the culture medium after 15 min, which is the necessary time for the Matrigel to reach its gelling state. Then, incubate each culture chamber under standard culture conditions.

Experimental treatment

- 3 Before performing experimental treatments, in order to reduce any Fetal Bovine Serum (FBS) interference on cellular proliferation, maintain the 3D HTMC cultures for at least 24 hrs in low and high glucose DMEM (1:1 mix), 2mM L-glutamine, 0.5% gentamicin and 100µg/ml streptomycin, w/o (FBS), according to Keller et al., (2018) Experimental Eye Research. 2018;171: 164–173. doi:10.1016/j.exer.2018.03.001. Then, proceed with experimental conditions.

3D HTMCs recovery

- 4 Release 3D-HTMCs from Matrigel at the end of experimental treatments with Corning® Cell Recovery Solution, following the "RECOVERY OF CELLS FROM CORNING MATRIGEL MATRIX" protocol, provided by Corning. 2h
Then, process the samples for further molecular analysis.