Sep 07, 2019

# HTMCs 3D culture method using Corning Matrigel Matrix

PLOS One

Stefania Vernazza[1,2]

[1]IRCCS, Fondazione G.B. Bietti, Rome, Italy, [2]

1 Works for me dx.doi.org/10.17504/protocols.io.574g9qw

Stefania Vernazza

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0221942

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Vernazza S, Tirendi S, Scarfì S, Passalacqua M, Oddone F, Traverso CE, Rizzato I, Bassi AM, Saccà SC (2019) 2D- and 3D-cultures of human trabecular meshwork cells: A preliminary assessment of an in vitro model for glaucoma study. PLoS ONE 14(9): e0221942. doi: 10.1371/journal.pone.0221942

## Cell Cultures

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

Maintain the HTMCs at 37◦C in a humidified atmosphere containing 5% $CO_2$, according to the manufacturer's indications. Sub-cultured HTMCs by TripLE$^{TM}$ Express (Invitrogen Life Technologies) treatment when the original flask is approximately 75% confluent.

## Cells embedded

24h

2 Slightly centrifuge 2.5 x 10 $^5$/cm$^3$ cells for 5 min at 90 rcf, descard the supernatant and gentle resuspend the cell pellet in undiluted Matrigel™ at 4°C, following the application of this formula:

Volume of added Matrigel (ml)/culture chamber growth area (cm$^2$).
i.e.: 0.2 ml /[(1.77/2)$^2$ 3.14 ] = 0.113 cm --> 1.13 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 culturesfor 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

2h

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, providing by Corning.
Then, process the samples for further molecular analysis.