Differentiation of endothelial cells on matrigel matrix

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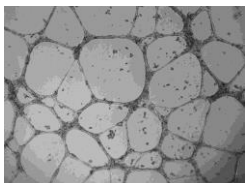
Evercyte Protocol No.:	Diff-matrigel-V3
Cells:	E.g. HUVEC/TERT2, HUVEC/TERT66, HDMVEC/TERT164, primary endothelial cells
Reagents and material:	PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at RT) Endothelial cell growth medium (e.g. EGM™ Endothelial Cell Growth Medium BulletKit™ supplemented with FBS and G418 when differentiating HUVEC/TERT2 cells), stored at 4°C Matrigel matrix (BD Corning, Cat# 354277, ready-to-use, stored aliquoted at -80°C) 24-well plate (tempered to 4°C)
Seeding of cells and induction of differentiation:	- thaw matrigel on ice (1 x 130 µl / sample to be tested) --> take out 3-4 aliquots - as soon as the matrigel has thawed mix 130 µl with 170 µl of cell culture medium while working on ice and carefully pipette the matrigel - medium mix into one well of a 24-well plate - avoid air bubbles! - put the plate at 37°C until the matrix solidifies - in the meantime, detach the cells from the culture vessel by using PBS and Trypsin-EDTA as described in the respective *Protocol for Passaging of Cells* - count the detached cell suspension using a hemocytometer - pipette a cell suspension containing 5 x 10^4 cells into a 1.5 ml sterile tube (Eppendorf) - centrifuge at 180 g for 10 min - remove the supernatant completely, resuspend the pellet in the remaining droplet - add 80 µl of cell culture medium - carefully pipette the cell suspension onto the matrigel - matrix (into the centre of the plate) - incubate at 37°C for at least 6 hours - interpretation: perform a microscopic evaluation, differentiated endothelial cells form typical tubule-like structures on matrigel (40x and 100x magnification)



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