In vitro Angiogenesis Assay: Endothelial Cell Proliferation, EdU Pulse Chase

1. Add 9,000 cells in 120µl EGM-2(MV) +growth factors –FBS in a 96 well plate. Add 30ul plasma to each well.
    120µl of 1:6 Matrigel suspension (45,000 cells/100µl)

2. Allow to recover at 37ºC for 18 hours
3. Add 120ul of 2X (20µM) EdU (Component A), incubate 6 hours
4. Remove media and add 100µl of 3.7% **formaldehyde** in PBS. Incubate for 15 minutes at room temperature.
5. Remove fixative and wash each well twice with 300µl PBS
6. Remove wash solution and add 100 µl of 0.1 Triton X-100 in PBS to each well. Incubate for 15 minutes at room temperature
7. Dilute 10X Click-iT EdU buffer in water.
8. Prepare Click-iT reaction cocktail
    In order, add
        10.3mL 1X Click-iT EdU reaction buffer
        480µl $CuSO_4$ (Componenet D)
        30µl Alexa Fluor azide (Componenent B)
        1.2 ml 1x Click-iT EdU buffer additive
9. Remove Triton X-100, and wash 2 x 300µl PBS.
10. Add 100µl Click-iT reaction cocktail per well
11. Incubate for 30 minutes at room temperature. Protect from light.
12. Remove the reaction cocktail and wash once with 100µl Click-iT reaction rinse buffer (Component F). Then remove the Click-iT reaction rinse buffer, and add 150µl PBS
13. Image.

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