Endothelial Cell Subculture and Preservation for *in vitro* Angiogenesis Assays

Part I: Subculture and Cryopreservation

1. Trypsinize
    a. Add 2ml of Trypsin (Lonza for HMVEC, TrypLE for HUVEC), incubate until cells lift

2. Neutralize
    a. Add 5 ml Trypsin Neutralizing Solution
    b. Rinse flask with ~5 HEPES-BSS

3. Pellet cells
    c. Centrifuge at 200g for 5 minutes

2. Aspirate supernatant and resuspend in EBM (for assays), EGM (to passage) or Cryopreservation media (Add DMSO after resuspension)

Part II: Cryopreservation

1. Aspirate supernatant and resuspend in 4ºC cryopreservation media
    a. <u>Cryopreservation Media</u>
        80% Complete growth media, EGM-2
        10% FBS
        10% DMSO
    b. Add DMSO only after resuspension of cells in Cryopreservation Media

2. Freeze 1ml aliquots of 500,000-2,000,000 cells per ml at -80C overnight, then liquid nitrogen for long term storage.

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