

Title

Preparation of hCMEC/D3 medium

Protocol

Materials:

- Fetal calf serum (FCS) (Cat. No.: 2-01F10-I, BioConcept)
- Penicillin/streptomycin (P/S) (Cat. No.: 15240-062, Gibco, Life Technologies)
- Hydrocortisone (Cat. No.: H0135, Sigma-Aldrich)
- Acid ascorbic (Cat. No.: A4544, Sigma-Aldrich)
- Chemically defined lipid concentrate (cat. No.: 11905031, Thermo fisher Scientific)
- HEPES (Cat. No.: 5-31F00-H, BioConcept)
- bFGF (Cat. No.: F0291, Sigma-Aldrich)
- EBM-2 medium (cat. No.: CC-3156, Lonza)

Procedure:

Complete medium EBM-2 for hCMEC/D3:

1. add 25 ml FCS (5%)
2. add 5 ml P/S (1%)
3. dissolve 1mg of hydrocortisone in 1 ml ethyl alcohol absolute, then add 250 ul of the solution to the EBM-2 medium
4. create a 1mg/ml acid ascorbic solution using sterile water, then add 2,5 ml to the EBM-2 medium
5. add 5ml of chemically defined lipid concentrate
6. add 5ml HEPES
7. add 2,5 ml of bFGF