INTRODUCTION

BBB^{Boost} is a specialized supplement designed to enhance barrier integrity and increase vessel perfusability in human BBB models. It strengthens barrier properties, stabilizes microvascular structures, and reduces variability across different cell lots. This protocol outlines the preparation of BBB^{Boost} working solution for cell culture applications.

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PREPARATION OF MEDIUM B WITH BBB^{Boost} ⏱ TIMING 30 MIN

MATERIALS

Reagents

- 100X BBB^{Boost}
- Medium B: EGM-2MV enriched with 1% v/v Astrocyte Growth Supplement (See REAGENT SETUP)

Others

- idenTx 40 plate seeded with cells in the gel and side channel
- 0.22 µm syringe filter
- Syringe

1. Add 10 µl of BBB^{Boost} (100X) per ml of medium B to achieve a final concentration of 1X BBB^{Boost}.

Reminder *Prepare extra to account for loss during sterile filtering.*

2. Sterile filter the medium using a 0.22 µm syringe filter.

3. Change the medium accordingly.

REAGENT SETUP

GROWTH FACTOR-ENRICHED MEDIA

Reagents

- $VEGF_{165}$ (R&D Systems, Cat. No. 293-VE-500)
- BSA (Sigma Aldrich, Cat. No. A9418)
- Astrocyte Growth Supplement (ScienCell, Cat. No. 1852)
- 1X PBS (Life Technologies, Cat. No. 10010023)
- EGM-2MV Microvascular Endothelial Cell Growth Medium-2 (Lonza, Cat. No. CC-3202)

4. Reconstitute recombinant human $VEGF_{165}$ at 100 µg/ml in sterile PBS containing at least 0.1% BSA. Aliquot it into smaller volumes and store them at -20°C.

5. **Medium A**: Add 0.5 µl of VEGF (100 µg/ml) and 10 µl of Astrocyte Growth Supplement (100X) per ml of medium to get cell culture medium enriched with 50 ng/ml VEGF and 1% v/v Astrocyte Growth Supplement.

6. **Medium B**: Add 10 µl of Astrocyte Growth Supplement (100X) per ml of medium to get cell culture medium enriched with 1% v/v Astrocyte Growth Supplement.

7. Reconstitute recombinant human $VEGF_{165}$ at 100 µg/ml in sterile PBS containing at least 0.1% BSA. Aliquot it into smaller volumes and store them at -20°C.