

Complete RPMI 1640 Medium for HBMEC (400ml)

HBMEC; Human Brain Microvascular Endothelial Cells

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|-----------------------------------|-------------------------------|
| - Penn-Strep (100 x) | 4 ml |
| - Na-Pyruvate (100 x) | 4 ml |
| - Nonessential Amino Acid (100 x) | 4 ml |
| - Vitamin mixtures (100 x) | 4 ml |
| - Heparin | 6,000 U / 50 ml RPMI 1640 |
| - ECGS (125 x) | 3.2 ml (2 vials: 1.6 ml/vial) |
| - FBS | 40 ml (Final conc.: 10 %) |
| - NuSerum | 40 ml (Final conc.: 10 %) |
| - RPMI 1640 with glutamine | adjust to 400 ml |

* Experimental media: FBS- and NuSerum-free

* Heparin: 6,000 U/400 ml (Final conc.: 15 U/ml media) – Filter sterilization

* ECGS: 100 mg/26.7 ml HBSS (Final conc.: 30 µg/ml media)

* Store at 4 °C

[Split ratio – 1:8]



Complete DMEM Medium for Mouse BMEC (400ml)

Mouse Brain Microvascular Endothelial Cell (BMEC)

[bEnd.3 cell line; ATCC CRL-2299]

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|----------------------|---------------------------|
| - Penn-Strep (100 x) | 4 ml |
| - FBS | 40 ml (Final conc.: 10 %) |
| - DMEM | adjust to 400 ml |

* Experimental media: 5% FBS

* DMEM: Mediatech #10-017-CV

* Store at 4 °C

[Split ratio – 1:3]



Complete 1:1 Ham's F10-MEM Medium for RBE4 (400ml)

RBE4; Rat Brain Microvascular Endothelial Cells

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|----------------------|-------------------------------|
| - Penn-Strep (100 x) | 4 ml |
| - bFGF (1,000 x) | 0.4 ml (Final conc.: 1 ng/ml) |
| - FBS | 40 ml (Final conc.: 10 %) |
| - L-Glutamine | 2 ml |
| - HEPES | 5 ml |
| - 1:1 Ham's F10-MEM | adjust to 400 ml |

* Experimental media: 5% FBS

* Ham's F-10: Mediatech #10-070

* MEM: Mediatech #15-010 (EMEM)

* bFGF: basic fibroblast growth factor, human recombinant (stock soln.: 1 µg/ml)

* L-Glutamine, 200 mM Solution: Mediatech #25-005-CI

* HEPES, 1 M Solution: Mediatech #25-060-CI

* Store at 4 °C

[Split ratio – 1:8]



Complete DMEM Medium for BMEC (400ml)

BMEC; Bovine Brain Microvascular Endothelial Cells

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|----------------------------------|-------------------------------|
| - bFGF (1,000 x) | 0.4 ml (Final conc.: 1 ng/ml) |
| - Heparin | 20 mg / 50 ml DMEM |
| - Antibiotic-Antimycotic (100 x) | 4 ml |
| - FBS | 16 ml (Final conc.: 4 %) |
| - Horse Serum | 60 ml (Final conc.: 15 %) |
| - DMEM | adjust to 400 ml |

* Experimental media: FBS- and horse serum-free

* Heparin: 20 mg/400 ml (Final conc.: 50 µg/ml media) – Filter sterilization

* bFGF: basic fibroblast growth factor, human recombinant (stock soln.: 1 mg/ml)

* Store at 4 °C

[Split ratio – 1:3]



Complete MCDB 131 Medium for HMEC-1 (400ml)

HMEC-1: Human Dermal Microvascular Endothelial Cells

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|-------------------------------------|------------------|---------------------|
| - Antibiotic/antimycotic (100 x) | 4 ml | |
| - L-Glutamine (20 x) | 20 ml | [final 10 mM] |
| - Hydrocortisone (1,000 x) | 0.4 ml | |
| - Epidermal Growth Factor (1,000 x) | 0.4 ml | |
| - FBS | 40 ml | (Final conc.: 10 %) |
| - MCDB 131 | adjust to 400 ml | |

* Basal Media: MCDB 131 (MediaTech #15-100-CV)

* Experimental media: 5% FBS

* Hydrocortisone (1,000 x), 1 mg/ml in ethanol

* Epidermal Growth Factor (EGF, Human) (1,000 x), 10 µg/ml in 10 µM acetic acid and 0.1% BSA

* Store at 4 °C

[Split ratio – 1:6]



Complete M199 Medium for HUVEC (400ml)

HUVEC; Human Umbilical Cord Vascular Endothelial Cells

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|----------------------------------|-------------------------------|
| - Penn-Strep (100 x) | 4 ml |
| - Antibiotic-Antimycotic (100 x) | 4 ml |
| - Na-Pyruvate (100 x) | 4 ml |
| - Heparin | 21,720 U / 40 ml M199 |
| - HEPES | 10 ml |
| - ECGS (125 x) | 3.2 ml (2 vials: 1.6 ml/vial) |
| - FBS | 80 ml (Final conc.: 20 %) |
| - M199 | adjust to 400 ml |

* Experimental media: 10% FBS

* Heparin: 21,720 U/400 ml (Final conc.: 54.3 U/ml media) – Filter sterilization

* HEPES, 1 M solution (Final conc.: 25 mM)

* ECGS: 100 mg/20 ml HBSS (Final conc.: 40 µg/ml media)

* Store at 4 °C

[Split ratio – 1:3]



Complete DMEM Medium for Mouse Microglial Cells [BV-2] (400ml)

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|----------------------|--------------------------|
| - Penn-Strep (100 x) | 4 ml |
| - FBS | 20 ml (Final conc.: 5 %) |
| - DMEM | adjust to 400 ml |

- * Experimental media: FBS-free
- * DMEM: Mediatech #10-017-CV
- * Store at 4 °C

[Split ratio – 1:6]



Complete DMEM Medium for Rat C6 Glioma Cell Line (400ml)

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|----------------------|---------------------------|
| - Penn-Strep (100 x) | 4 ml |
| - FBS | 40 ml (Final conc.: 10 %) |
| - DMEM | adjust to 400 ml |

- * Experimental media: FBS-free
- * DMEM: Mediatech #10-017-CV
- * Store at 4 °C

[Split ratio – 1:6]



Complete MEM Medium for Rat Primary Astrocytes (400ml)

Rat Primary Astrocytes

- | | |
|----------------------------------|---------------------------|
| - Antibiotic/Antimycotic (100 x) | 4 ml |
| - L-Glutamine (100 x) | 4 ml |
| - Horse serum | 40 ml (Final conc.: 10 %) |
| - MEM | adjust to 400 ml |

* MEM (MediaTech #15-010-CV)

* Experimental media: 5% FBS

* L-Glutamine, 200 mM Solution: Mediatech #25-005-CI

* Store at 4 °C

[Split ratio – 1:3]



Complete RPMI 1640 Medium for THP-1 Monocytes (400ml)

THP-1 Monocytes

- Penn-Strep (100 x) 4 ml (Final conc: 1%)
- FBS 40 ml (Final conc.: 10 %)
- RPMI 1640 with glutamine adjust to 400 ml

* Experimental media: FBS- free

* Store at 4 °C

Monday, Wednesday, Friday: Replace media

Centrifuge [800 x g, 5 min, RT]

Resuspend in 1 ml media

When cell # = 10×10^6 cells/ml → pass into 2 T75 flasks

Experiment days:

Centrifuge [800 x g, 5 min, RT]

Wash in 10 ml experimental media

Centrifuge [800 x g, 5 min, RT]

Resuspend in RPMI + 1% PS + 3% FBS



Complete DMEM Medium for MDA-MB-231 & MCF-7 (400ml)

Human Breast Cancer Cell Line (MDA-MB-231: Highly Metastatic)

- Penn-Strep (100 x) 4 ml
- FBS 40 ml (Final conc.: 10 %)
- DMEM w/ glutamine and high glucose adjust to 400 ml

* Experimental media: FBS-free DMEM

* Store at 4 °C

[Split ratio – 1:3]

