

# Differentiation of Human Induced Pluripotent Stem Cells to Brain Microvascular Endothelial Cell-Like Cells with a Mature Immune Phenotype

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## Materials

| Name                                                             | Company                   | Catalog Number | Comments |
|------------------------------------------------------------------|---------------------------|----------------|----------|
| 0.22 mm Syringe filter                                           | TPP                       | 99722          |          |
| 15 mL Centrifuge tube                                            | Falcon                    | 352196         |          |
| 40 µm Falcon cell strainer                                       | Falcon                    | 352340         |          |
| 5 mL Round-bottom tube                                           | SPL                       | 40005          |          |
| 50 mL Centrifuge tube                                            | Falcon                    | 352070         |          |
| 96-Well plate, round bottom                                      | SPL                       | 34096          |          |
| Accutase                                                         | Sigma-Aldrich             | A6964-500ml    |          |
| Acetic acid                                                      | Sigma-Aldrich             | 695092         |          |
| Advanced DMEM/F12                                                | Life Technologies         | 12634          |          |
| All-in-One Fluorescence Microscope                               | Keyence                   | BZ-X810        |          |
| B-27 Supplement (503), serum free                                | Thermo Fischer Scientific | 17504044       |          |
| Bovine serum albumin (BSA), 7.5% in dPBS                         | Sigma-Aldrich             | A8412          |          |
| CellAdhere Dilution Buffer                                       | STEMCELL Technologies     | ST-07183       |          |
| CellTracker Green CMFDA Dye                                      | Invitrogen                | C7025          |          |
| Chamber slides                                                   | Thermo Fischer Scientific | 178599         |          |
| CHIR99021                                                        | Selleck Chemicals         | S1263          |          |
| Collagen IV from human placenta                                  | Sigma-Aldrich             | C5533          |          |
| Corning tissue culture plates (12-well)                          | Corning                   | 3512           |          |
| Corning tissue culture plates (6-well)                           | Corning                   | 3506           |          |
| Cryo tube innercap 2.0 mL                                        | Watson                    | 1396-201S      |          |
| Dimethylsulfoxide (DMSO), sterile                                | Sigma-Aldrich             | D2650          |          |
| DMEM (13), [+], 4.5 g/L D-glucose, [-] L-glutamine, [-] pyruvate | Thermo Fischer Scientific | 31053-028      |          |

|                                                                     |                           |             |  |
|---------------------------------------------------------------------|---------------------------|-------------|--|
| Dulbecco's (d) PBS (without calcium, magnesium)                     | Thermo Fisher             | 14190250    |  |
| Dulbecco's modified Eagle's medium/nutrient mixture F-12 (DMEM-F12) | Thermo Fischer Scientific | 11320074    |  |
| EasySepFITC Positive Selection Kit II                               | STEMCELL Technologies     | 18558       |  |
| EasySepMagnet                                                       | Stemcell Technologies     | 18000       |  |
| Ethylenediaminetetraacetic Acid Solution 0.02% in DPBS              | Sigma                     | E8008-100ML |  |
| Fetal Bovine Serum, qualified                                       | Thermo Fischer Scientific | 10270106    |  |
| Fibronectin from bovine plasma                                      | Sigma-Aldrich             | F1141       |  |
| Ficoll-Paque PLUS                                                   | Sigma-Aldrich             | GE17144002  |  |
| FIJI software (Version 2.0.0)                                       | Image J, USA              |             |  |
| FlowJo version10                                                    | BD                        |             |  |
| Fluorescein sodium salt                                             | Sigma-Aldrich             | F6377       |  |
| GlutaMAX Supplement                                                 | Thermo Fischer Scientific | 35050-061   |  |
| Glutaraldehyde solution                                             | Sigma-Aldrich             | G6257       |  |
| HCL                                                                 | Sigma-Aldrich             | H1758       |  |
| HEPES buffer solution                                               | Thermo Fischer Scientific | 15630-056   |  |
| Human Endothelial Serum Free Medium (hESFM)                         | Thermo Fischer Scientific | 11111-044   |  |
| Human fibroblast growth factor 2 (FGF2)                             | Tocris                    | 233-FB-500  |  |
| iPS human induced pluripotent stem cells                            | Riken RBC                 | HPS1006     |  |
| Kanamycin Sulfate (100x)                                            | Thermo Fischer Scientific | 15160-047   |  |
| L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate            | Sigma-Aldrich             | A8960-5G    |  |
| L-Glutamine 200 mM (1003)                                           | Thermo Fischer Scientific | 25030-024   |  |
| Matrigel, growth factor reduced                                     | Corning                   | 354230      |  |
| MEM NEAA (1003)                                                     | Thermo Fischer Scientific | 11140-035   |  |
| Methanol                                                            | Sigma-Aldrich             | 32213       |  |
| Mowiol                                                              | Sigma-Aldrich             | 81381       |  |
| mTeSR Plus-cGMP                                                     | STEMCELL Technologies     | ST-100-0276 |  |
| mTeSR1 complete kit (basal medium plus 53 supplement)               | STEMCELL Technologies     | 85850       |  |
| NaCl                                                                | Sigma-Aldrich             | 71376       |  |
| Paraformaldehyde                                                    | Millipore                 | 104005      |  |
| Pen Strep                                                           | Thermo Fischer Scientific | 15140-122   |  |
| Recombinant Human IFN-gamma Protein                                 | R&D Systems               | 285-IF-100  |  |
| Recombinant Human IL-2                                              | BD Biosciences            | 554603      |  |
| Recombinant Human TNF-alpha Protein                                 | R&D Systems               | 210-TA-020  |  |
| ROCK inhibitor Y-27632                                              | Tocris                    | 1254        |  |
| RPMI medium 1640                                                    | Thermo Fischer Scientific | 21875-034   |  |
| Scalpel                                                             | FEATHER                   | 2975-11     |  |
| Skim milk                                                           | BD Biosciences            | 232100      |  |
| Sodium azide (NaN3)                                                 | Sigma-Aldrich             | 71290       |  |
| Sodium pyruvate                                                     | Thermo Fischer Scientific | 11360-039   |  |
| Transwells, PC Membrane, 0.4 mm, 12 mm, TC-Treated                  | Corning                   | 3401        |  |

|                              |                       |        |  |
|------------------------------|-----------------------|--------|--|
| Tris base                    | Sigma-Aldrich         | 93362  |  |
| Triton X-100                 | Sigma-Aldrich         | X100   |  |
| Vitronectin XF               | STEMCELL Technologies | 078180 |  |
| Water, sterile, cell culture | Sigma-Aldrich         | W3500  |  |