INSTRUCTION SHEET

PureStem® Choroid Plexus Cell Differentiation Kit

Catalog Number: EM-2006

Kit Constituents:	Part number
PureStem HyGlu Basal DM, 242 mL	EM-2101
Pen/Strep, 2.5 mL	EM-1414
L-alanine-L-glutamine, 2.5 mL	EM-1415
Pyruvate, 2.5 mL	EM-1416
Proline plus ITS, 0.5 mL	EM-1417
2-phospho-L-ascorbic acid, 250 µL	EM-1408
Dexamethasone, 250 µL	EM-1409
BMP-4, 130 µL	EM-1411

FOR RESEARCH USE ONLY

The PureStem Choroid Plexus Cell Differentiation Kit, when used with PureStem ES-210, Ecto-ntu Progenitor (Ectodermal-neural tube progenitor), PureStem Progenitor E68 and PureStem Progenitor E69 causes these progenitors to upregulate TTR (transthyretin) expression -through exposure to BMP-4 in micromass culture. PureStem ES-210, Ecto-ntu Progenitor expresses markers of brain and nervous system related cells (TTR, ZIC2, TH and NES). Choroid plexus cells line the choroid plexus, a structure in the ventricles of the brain, where they function to produce cerebrospinal fluid. Always handle human sourced materials at biological safety level two.

PREPARATION OF PURESTEM DIFFERENTIATION MEDIUM

Preliminary PureStem Differentiation Medium - Working in a sterile field, supplement the PureStem HyGlu Basal medium (EM-2101) with the components listed below. Decontaminate the outside surface of each vial and the medium bottle with ethanol or isopropanol before supplementation. Rinse vials with medium to ensure the total content is used. Filter the supplemented medium through 0.22 µm unit filter and record the date of supplementation. Supplemented medium can be stored at 2-8°C for up to four weeks.

2.5 mL EM-1414 (Pen/Strep)
2.5 mL EM-1415 (L-alanine-L-glutamine)
2.5 mL EM-1416 (Pyruvate)
0.5 mL EM-1417 (Proline plus ITS)
250 µL EM-1408 (2-phospho-L-ascorbic acid)
250 µL EM-1409 (Dexamethasone)

Preparation of Final Differentiation Medium - Immediately before use and in a sterile field using aseptic technique, add EM-1411 (BMP-4) to the desired volume needed to feed cells according to the instructions below. Always add supplements in a sterile field and always decontaminate the outside of the vials using ethanol or isopropanol before opening.

Add 1µL of EM-1411 to each 2 mL of Preliminary PureStem Differentiation Medium to prepare Final Differentiation Medium. We recommend that you use approximately 4 mL for each well of a 6-well plate or 15 mL for feeding cells in a 10 cm dish (assuming cell numbers are approximately $1x10^6$ and $6x10^6$ cells respectively).

NOTE: The first time EM-1411 (BMP-4) is thawed, aliquot into separate labeled sterile microfuge tubes (or equivalent) and freeze at ≤-65°C for future or near-term use. Prepare aliquots on the basis of predicted quantities of Final Differentiation Medium that will be used during each feed.

HARVESTING CELLS FOR DIFFERENTIATION:

NOTE: See Product Information Sheet provided with specific PureStem progenitor for recommended culture conditions and growth medium.

Steps 1-6 should be performed in a sterile field. Decontaminate the outside of containers before opening using ethanol or isopropanol.

1. Expand cells in appropriate growth medium according to the instructions provided to provide sufficient cell numbers for the preparation of multiple 10 µL micromasses containing 200,000 cells each.
2. When cell number is sufficient, remove the growth medium from culture ware by aspiration. Wash cells with PBS (Ca & Mg free), THIS IS A CRITICAL STEP because growth medium contains proteins and Ca that can inactivate trypsin. Detach cells by covering with trypsin/EDTA (0.25% trypsin diluted 1/3 with PBS i.e. 1 part trypsin in 2 parts PBS). Place in incubator and check for cell detachment every few minutes. Allow trypsin to work until approximately 90% of the cells are rounded up. DO NOT OVERTRYPSINIZE as it can damage the cells. Look for cell detachment – it may be necessary to lightly rap the flask to detach the cells.
3. Following cell detachment (i.e. under 10 minutes), inactivate the trypsin by adding serum-containing growth media (at least 5X the volume of diluted trypsin used). Place the cell suspension into a sterile container or polypropylene tube.
4. Determine the cell number and concentration using a Coulter counter (Beckman Coulter Z2 Cell and Particle Counter or equivalent), hemocytometer or equivalent device.
5. Place the appropriate volume containing the desired cell number (i.e. 20 mL with 20 x 10^6 cells), into a separate sterile polypropylene 50 mL tube(s).
6. Centrifuge at 150 x g at room temperature for 5 min to pellet cells. See "Preparing 10 µL micromasses seeded in growth media" below for further instructions.

PREPARING 10 µL MICROMASSES SEEDED IN GROWTH MEDIA:

Perform steps in a sterile field. Decontaminate the outside of containers before opening using ethanol or isopropanol.

1. Remove the supernatant (medium) by aspiration, and gently resuspend cell pellet in the appropriate volume of growth medium to provide a final cell concentration of 20x10^6 cells/mL. For example, for 20x10^6 cells the volume is brought up to 1 mL with growth medium. Pipet up and down gently to ensure cells are evenly distributed. Note that 1 mL of cell suspension will yield 100 micromasses.
2. Using a P20 or equivalent pipet and taking care not to touch the pipet tip to the dry surface of the culture ware, evenly space 10 µL aliquots (each containing 200,000 cells) onto a tissue culture treated polystyrene dish(es) (10 cm diameter) or in wells of a 6-well plate.
3. Place the seeded dish into the humidified incubator (37°C, 5% O_2, 10% CO_2) for 1.5-2.5 hours to allow cell attachment.
4. After attachment, slowly add growth medium (i.e. 15 mL to a 10 cm dish) taking care not to disturb attached cell micromasses. Return the dish back into the incubator.
5. The following morning prepare Final Differentiation Medium by adding 1 µL EM-1411 (BMP-4) to every 2 mL of Preliminary Differentiation Medium needed for feeding.
6. Gently aspirate off growth medium and wash with PBS (Ca Mg free).
7. Add appropriate volume of Final Differentiation Medium. For example, for feeding a 10 cm dish 15 mL is the recommended volume.
8. Feed cells 3X/week (i.e. Monday, Wednesday, and Friday) by aspirating old medium and gently adding freshly prepared Final Differentiation Medium until micromasses are harvested 14 days after the first feeding with Final Differentiation Medium.

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