

## Poietics™ Normal Human Dendritic Cell System NHDC – Technical Information & Instructions

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### I. Introduction

The Poietics™ human dendritic cell system contains primary derived cultures of normal human dendritic cells (NHDC). The system includes optimized medium for cell sustenance. This system may be used for experimental applications such as: effectiveness of immunosuppressive drugs to alter dendritic cell cytokine secretion, antigen presentation studies, antigen uptake studies; phenotypic studies of NHDC. Poietics™ normal human dendritic cell system is convenient and easy to use, freeing the researcher to focus on results. Cryopreserved NHDC are shipped frozen.

Poietics™ cells, medium and reagents are quality tested together and guaranteed to perform as a complete cell system.

### II. Cell Culture System Components (Sold Separately)

- One normal human dendritic cell product – (cryopreserved)
- One LGM-3™ Lymphocyte Growth Medium-3 Medium - 500 ml (Lonza Catalog No. CC-3211)
- Recombinant Human Granulocyte-macrophage colony-stimulating factor (GM-CSF) (R&D Systems Catalog No. 215-GM-010, or similar)
- Recombinant Human Interleukin 4 (IL-4) (R&D Systems Catalog no. 204-IL-010, or similar)

### III. General Cell Information

| Cat. No. | Description                  | Recommended Culture Media | Cryopreserved Passage Number | Seeding Density Upon Thaw    | Time to Differentiation |
|----------|------------------------------|---------------------------|------------------------------|------------------------------|-------------------------|
| CC-2701  | Normal Human Dendritic Cells | LGM-3™ Medium             | Passage 0                    | 40,000 cells/cm <sup>2</sup> | 4 days                  |

### IV. Quality Control

| Cat. No. | Description                  | Cells/Vial              | Viable Time in Culture (w/ cytokines) | Characterization   |
|----------|------------------------------|-------------------------|---------------------------------------|--|
| CC-2701  | Normal Human Dendritic Cells | ≥3,000,000 viable cells | 7 days                                | CD11c+, CD14-<br>CD86 (FIO*), CD80 (FIO*), HLA-DR (FIO*) |

All cells are performance assayed and test negative for HIV-1, mycoplasma, Hepatitis-B, Hepatitis-C, bacteria, yeast and fungi. Poietics™ Media are formulated for optimal growth of specific types of human cells. Certificates of Analysis (COA) for each cell strain are shipped with each order. COAs for all other products are available upon request. Please see Section XIV (Product Warranty, Page 5) for more information on Quality Control claims and guarantees.

\*For Information Only (FIO)

### V. Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells: Remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact Customer Service.
3. LGM-3™ Medium instructions: store medium at 2°-8°C. Do not freeze.
4. Store Recombinant Human Granulocyte-macrophage colony-stimulating factor (GM-CSF) and Recombinant Human Interleukin 4 (IL-4) at ≤-20°C in a freezer that is not self-defrosting.

Using media or reagents other than what is recommended will void the cell warranty. Please contact Scientific Support if you need help selecting media and/or reagents.

### VI. Preparation of Culture Media

1. Decontaminate external surfaces of all vials and the medium bottle with ethanol or isopropanol.
2. To formulate complete dendritic cell culture medium, aseptically transfer 200 ml of LGM-3™

Medium into a sterile container. Transfer 10 µg of Recombinant Human Granulocyte-macrophage colony-stimulating factor (GM-CSF) to the 200 ml aliquot of LGM-3™ Medium for a final GM-CSF concentration of 50 ng/ml. Transfer 10 µg of Recombinant Human Interleukin 4 (IL-4) to the 200 ml aliquot of LGM-3™ Medium for a final IL-4 concentration of 50 ng/ml. Stir until all cytokines are dissolved.

3. After cytokines are added to basal medium, store at 2°-8°C and use within 1 month. Do not freeze medium.

**NOTE:** If there is concern that sterility was compromised during the supplementation process, the entire newly prepared culture medium may be re-filtered with a 0.2 µm filter to assure sterility. Routine re-filtration is not recommended.

### VII. Thawing of Cells / Initiation of Culture Process

1. The recommended seeding density when initially thawing NHDC from cryopreservation is 40,000 cells/cm<sup>2</sup>. One ampoule of NHDC containing ≥3,000,000 cells contains enough cells to plate at least one T-75 flasks.

**NOTE:** Alternate flask/well sizes can be utilized as long as the appropriate seeding density is achieved.

2. To set up culture vessels, calculate the number of vessels needed based on the recommended seeding density and the surface area of the vessels being used. Add the appropriate amount of medium to the vessels (1 ml/5 cm<sup>2</sup>) and allow the vessels to equilibrate in a 37°C±1°C, 5% CO<sub>2</sub>, 90%±2% humidity incubator for at least 30 minutes.
3. Prior to thawing cells, allow 25 ml of complete dendritic cell culture medium (LGM-3™ Medium supplements with GM-CSF and IL-4) to equilibrate to room temperature.
4. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure and then retighten. Quickly thaw the cryovial in a 37°C water bath being careful not to submerge the entire vial. Watch your cryovial closely; when the last sliver of ice melts, remove it. Do not submerge it completely. Thawing the cells for longer than 2 minutes results in less than optimal results.
5. Using a micropipette, gently add the thawed cell suspension to a 50 ml sterile polypropylene centrifuge tube. Slowly dilute the cell suspension with room temperature complete dendritic cell culture medium to a final volume of 25 ml.
6. Centrifuge at 400 x g for 10 minutes at room temperature.
7. Carefully discard the supernatant and resuspend the pellet in 1 ml of medium using a micropipette. Dispense cells into the culture vessels set up earlier. Gently rock the culture vessel to evenly distribute the cells and return to the 37°C±1°C, 5% CO<sub>2</sub>, 90%±2% humidity incubator.

## VIII. Maintenance

1. After initially plating the cells, return vessels to the incubator for 4 days. After this point, cells are ready to use.

**NOTE:** After initially plating the cells, there is no need to change the media during the first four days.

2. If culturing cells for longer than 4 days, on Day 4, warm an appropriate amount of medium to 37°C in a sterile container. Remove the medium and replace it with the warmed, fresh medium and return the flask to the incubator.

3. Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer and warm only the required volume to a sterile secondary container.
4. Cells may be maintained for up to 7 days.

**NOTE:** Once plated, these cells cannot be subcultured or cryopreserved. Cells must be plated into their final research vessel and must be used within 7 days after thawing.

## IX. Ordering Information

### Cryopreserved Normal Human Dendritic Cells:

| Cat. No. | Product                      | Description             |
|----------|------------------------------|-------------------------|
| CC-2701  | Normal Human Dendritic Cells | ≥3,000,000 viable cells |

### Dendritic Cell Culture Media (Sold Separately):

| Cat. No. | Product       | Description                              |
|----------|---------------|--|
| CC-3211  | LGM-3™ Medium | 500 ml LGM-3™ Lymphocyte Growth Medium-3 |

Additional components are required for differentiation please see Section II (Cell Culture System Components, Page 1) for a complete listing of required components.

## X. Product Warranty

Cultures have a finite lifespan *in vitro*.

Lonza guarantees the performance of its cells in the following manner only if Poietics™ Media is used exclusively and the recommend protocols are followed. The performance of cells is not guaranteed if any modifications are made to the complete cell system.

When placing an order or for Scientific Support, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics™ Products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, additional information or want to speak with Scientific Support, you may contact Lonza by web, e-mail, telephone, fax or mail (See page 1 for details).

**THESE PRODUCTS ARE FOR RESEARCH USE ONLY.** Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

**WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS**

**POTENTIALLY INFECTIOUS.** Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-1, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, [Biosafety in Microbiological and Biomedical Laboratories](#), 5<sup>th</sup> ed. If you require further information, please contact your site safety officer or Scientific Support.

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