Lonza Walkersville, Inc.
Walkersville, MD 21793-0127 USA
U.S. Scientific Support: 800 521 0390
scientific.support@lonza.com
EU/ROW Scientific Support: +32 87 321 611
scientific.support.eu@lonza.com
Document # TS-CC-2701-2 08/16
www.lonza.com

Normal Human Dendritic Cells

NHDC – Technical Information & Instructions

Table of Contents:

I. Introduction

Lonza Normal Human Dendritic Cells (NHDC) are monocyte-derived, immature dendritic cells (DC). Through use of culture with specific cytokines, immature DCs have the ability to further differentiate into mature DCs.

LGM-3™ Medium supplemented with IL-4 and GM-CSF is recommended for maintenance of the immature phenotype. Additional cytokines are required for differentiation to the mature phenotype.

Every lot of Lonza NHDCs are tested for expression levels of HLA-DR, CD11c, CD86, CD80, and CD14.

Cell Surface Marker	Significance
HLA-DR	Class II MHC antigen – antigen presentation capabilities
CD11c	Adhesion molecule – DC-T cell adherence and T cell activation
CD86	Co-stimulatory molecule – early maturation marker
CD80	Co-stimulatory molecule – late maturation marker
CD14	Lineage marker – evidence of differentiation from monocytes

For answers to Frequently Asked Questions regarding these products, please visit our FAQ Database:
www.lonza.com/faq

For citations citing the use of these products, please visit our Citations Database:
www.lonza.com/citations

II. Required Reagents

(Components Sold Separately)

- Cryopreserved normal human dendritic cells (Lonza Catalog No. CC-2701)
- 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)

Lonza guarantees the performance of these cells only if appropriate media and reagents are used exclusively and the recommended storage and use protocols are followed. Any modifications made to the recommended cell systems, including the use of alternative media, reagents or protocols, will void cell and media performance guarantees. If you need assistance in selecting the appropriate media, reagents, or protocol, please contact Lonza Scientific Support.

III. General Cell Information

Cat. No.	Description	Recommended Culture Medium	Cryopreserved Passage Number	Recommended Seeding Density Upon Thaw
CC-2701	Normal Human Dendritic Cells	LGM-3™ Medium	Passage 0	40,000 cells/cm^2

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	HLA-DR (FIO*), CD11c (FIO*), CD86 (FIO*), CD14 (FIO*), CD80 (FIO*)

All cells are performance assayed and test negative for HIV-1, mycoplasma, Hepatitis-B, Hepatitis-C, bacteria, yeast and fungi. 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 XI (Product Warranty, Page 4) 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.

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 100 ml of LGM-3™ Medium into a sterile container. Transfer 80,000 units of Recombinant Human Granulocyte-macrophage colony-stimulating factor (GM-CSF) to the 100 ml aliquot of LGM-3™ Medium for a final GM-CSF concentration of 800 U/ml. Transfer 80,000 units of Recombinant Human Interleukin 4 (IL-4) to the 100 ml aliquot of LGM-3™ Medium for a final IL-4 concentration of 800 U/ml. Stir until all cytokines are dissolved.
3. After cytokines are added to basal medium, store at 2°-8°C and use within 1 week. 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

1. Prior to thawing cells, prepare a thawing medium by adding 10 ml of fetal bovine serum to 90 ml of incomplete dendritic cell culture medium (LGM-3™ Medium WITHOUT GM-CSF or IL-4) OR by adding 1 ml of bovine serum albumin (BSA) to 99 ml of incomplete dendritic cell culture medium (LGM-3™ Medium WITHOUT GM-CSF or IL-4). Allow thawing medium to equilibrate to room temperature.
2. 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℃ 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.
3. Using a micropipette, gently add the thawed cell suspension to a 50 ml sterile polypropylene centrifuge tube. Rinse the cryovial with 1.0 ml of thawing medium and add the rinse to the cell suspension drop by drop while gently swirling the tube. This step should take approximately 1 minute.
4. Slowly bring the total volume of cell suspension up to approximately 50 ml by adding room temperature thawing medium drop by drop, while gently swirling the tube. This step should take approximately 5-10 minutes.
5. Centrifuge at 200 x g for 15 minutes at room temperature.
6. Carefully remove all but approximately 1.0 ml of the supernatant by pipette. Resuspend the pellet in the remaining medium using a micropipette. Avoid excessive pipetting.
7. Transfer the cell suspension to a new 15 ml sterile polypropylene centrifuge tube. Rinse the previously used 50 ml polypropylene centrifuge tube with approximately 5 ml of thawing medium and add the rinse to the cell suspension slowly while gently swirling the tube.
8. Slowly bring the total volume of cell suspension up to approximately 15 ml by adding room temperature thawing medium slowly while gently swirling the tube.
9. Loosen the cap on the 15 ml polypropylene centrifuge tube and allow the cells to rest for 1 hour in a 37℃±1℃, 5% CO2, 90%±2% humidity incubator.
10. Centrifuge at 200 x g for 15 minutes at room temperature.
11. Carefully remove all but approximately 200 µl of the supernatant by pipette.
12. Dilute the cells to a final volume of 2 to 3 ml of complete dendritic cell culture medium (LGM-3™ Medium WITH GM-CSF and IL-4) and note the total volume of the diluted cell suspension. Resuspend the pellet in the medium using a micropipette. Avoid excessive pipetting.
13. Determine cell count and viability using a hemacytometer and Trypan Blue. Make a note of your cell yield for later use.
14. Further dilute the suspension complete dendritic cell culture medium (LGM-3™ Medium WITH GM-CSF and IL-4) to achieve a final cell density of 200,000 viable cells per ml. After dilution, re-count the cells to validate proper dilution.

VIII. Initiation of Culture Process

1. The recommended seeding density when initially plating NHDC is 40,000 viable cells/cm^2. One ampoule of NHDC containing ≥3,000,000 viable 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 as well as the surface area of the vessels being used.
3. Dispense 1 ml of the previously prepared cell suspension (at 200,000 viable cells per ml) per every 5 cm^2 of culture surface area in the culture vessels set up earlier. For example, add 15 ml of cell suspension at 200,000 viable cells per ml to one T-75 flask. Gently rock the culture vessel to evenly distribute the cells and return to the 37℃±1℃, 5% CO_2, 90%±2% humidity incubator.

IX. Maintenance

1. After initially plating the cells, return vessels to the incubator.
2. Change the growth medium 48 hours after seeding and every other day (every 48 hours) thereafter.
3. Warm an appropriate amount of medium to 37℃ in a sterile container. Remove the medium and replace it with the warmed, fresh medium and return the flask to the incubator.
4. 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.
5. 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.

X. 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:

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.

XI. Product Warranty

Cultures have a finite lifespan *in vitro.*

Lonza guarantees the performance of Poietics™ cells only if appropriate Poietics™ media and reagents are used exclusively and the recommended storage and use protocols are followed. Any modifications made to the recommended cell systems including the use of alternative media, reagents or protocols, will void cell and media performance guarantees. If you need assistance in selecting the appropriate media, reagents, or protocol, please contact Lonza Scientific Support.

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* diagnostic 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-I, 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, 5th ed. If you require further information, please contact your site safety officer or Scientific Support.