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Document # AA-1020-9 06/11

Poietics™ immune cell systems

Instructions for use

Safety statements

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-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 can not 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 edition. If you require further information, please contact your site safety officer or scientific support.

Unpacking and storage instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells – If there is dry ice left in the package, place cryovials immediately into liquid nitrogen. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact customer service.
3. Upon arrival, store LGM-3™ at 4-8°C.

Thawing of cells

1. The recommended density for use for NHDC is 2-5 x 10^4 cells/cm^2 and for HPBMC is 1-5 x 10^6 cells/ml.
2. To set up cultures calculate the number of vessels needed based on the recommended seeding density and the surface area of the vessels being used.
3. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure, then retighten. Quickly thaw the cryovial in a 37°C water bath for 1-2 minutes, being careful not to submerge the entire vial.

Initiation of culture process

Set up NHDC

1. To complete differentiation of NHDC, culture cells in LGM-3™ with the cytokines IL-4 (50 ng/ml) and GM-CSF (50 ng/ml) for 4 days.
2. Before thawing cells, equilibrate LGM-3™ to room temperature.
3. Transfer contents of cryovial to a 50 ml polypropylene centrifuge tube. Dilute slowly to 25 ml with LGM-3™.
4. Centrifuge at 400 x g for 10 minutes.
5. Aspirate supernatant medium and resuspend cells in LGM-3™ to an appropriate density for seeding at 2-5 x 10^4 cells/cm^2 .
6. Supplement with cytokines and seed the cultures.
7. Return the culture vessels to 37°C, 5% CO_2 incubator for 4 days. At this point the cells can be used or refed.

Set up HPBMC

1. Warm LGM-3™ or medium containing 10% FBS. Add DNase I (20 U/ml).
2. Aseptically transfer a maximum of 2 ml of cell suspension to a 50 ml conical tube.
3. Rinse the vial with 1 ml of medium. Add the rinse dropwise to the cells while gently swirling the tube.
4. Slowly add medium dropwise to the cells until the total volume is 50 ml, while gently swirling after each addition of medium.
5. Centrifuge the cell suspension at 200 x g at room temperature for 15 minutes.
6. Carefully remove by pipetting all but 2 ml of the wash. Gently resuspend the cell pellet in the remaining 2 ml of medium and count. The cells are now ready to be put into culture.
7. HPBMC can be cultured in LGM-3™. The addition of cytokines may be necessary based on application.

Set up $CD4^+$ T cells and $CD19^+$ B cells

1. Warm medium containing 10% FBS or 1% BSA. Quickly thaw the vial of frozen cells in a 37℃ water bath, about 1-2 minutes. Wipe the outside of the vial with 70% ethanol.
2. Aseptically transfer a maximum of 2 ml of cell suspension to a 50 ml conical tube. For 1 million cells or less, use a 15 ml conical tube.
3. Rinse the vial with 1 ml of medium. Add the rinse dropwise to the cells while gently swirling the tube (≈ 1 minute).
4. Slowly add enough medium dropwise to the cells until the total volume is 5 ml, while gently swirling after each addition of several drops of medium (≈ 3 minutes).
5. Slowly bring the volume up to fill the tube by adding 1 ml to 2 ml volumes of medium dropwise, while gently swirling after each addition of medium (≈ 5 to 10 minutes).
6. Centrifuge the cell suspension at 200 X g at room temperature for 15 minutes.
7. Carefully remove most of the wash by pipetting (and save in a second tube), leaving a few milliliters behind so the cell pellet is not disturbed. Gently resuspend the cell pellet in the remaining medium. If you are using a 50 ml tube, transfer the cells to a 15 ml conical tube and rinse the 50 ml tube with 5 ml of medium. Slowly add the 5 ml wash medium to the cell suspension with gentle swirling.
8. Slowly bring the volume up to fill the tube by adding 1 ml to 2 ml volumes of medium while gently swirling after each addition of medium.
9. Centrifuge the cell suspension at 200 X g at room temperature for 15 minutes.
10. Carefully remove all but 2 ml of the wash by pipetting. Gently resuspend the cell pellet in the remaining 2 ml of medium and count. If cell count is lower than expected, centrifuge wash saved in step 8 at a higher speed, count and combine if necessary.
11. Rest the cells for 1 hour at 37℃ and 5% CO_2. Count the cells a second time. The cells are ready to be put in culture.
12. The $CD4^+$ T Cells and $CD19^+$ B Cells can be cultured in LGM-3™. The addition of cytokines may be necessary based on application.

Ordering information

Cryopreserved cells (single donor)	
CC-2701	NHDC 2.5 x 10^6 cells
CC-2702	HPBMC
2C-200	Cord blood $CD4^+$ T cells
2W-200	Peripheral blood $CD4^+$ T cells
2C-300	Cord blood $CD19^+$ B cells

Related products

Immune cell media systems (must be purchased separately):

CC-3211	LGM-3™	Lymphocyte growth medium-3, 500 ml, serum-free, complete with albumin, insulin, transferrin and gentamicin

Product warranty

CULTURES HAVE A FINITE LIFESPAN *IN VITRO*. Lonza warrants its cells only if Poietics™ media and reagents are used.

1. The cryopreserved NHDC and HPBMC are provided as cryopreserved cells from primary cultures.
2. NHDC and HPBMC are cryopreserved in 86.5% IMDM, 7.5% DMSO, 4% human serum albumin and 2% hydroxy-ethyl-starch.

Quality control

Donors are screened for HIV-1, hepatitis B, hepatitis C, HTLV-1, HTLV- 2 and RPR. After four days in culture the NHDC stain ≥ 90% positive for CD11c and CD86 surface antigens, and are ≤ 5% positive for CD14 surface antigen using flow cytometry. For detailed information concerning QC testing, please refer to the certificate of analysis.