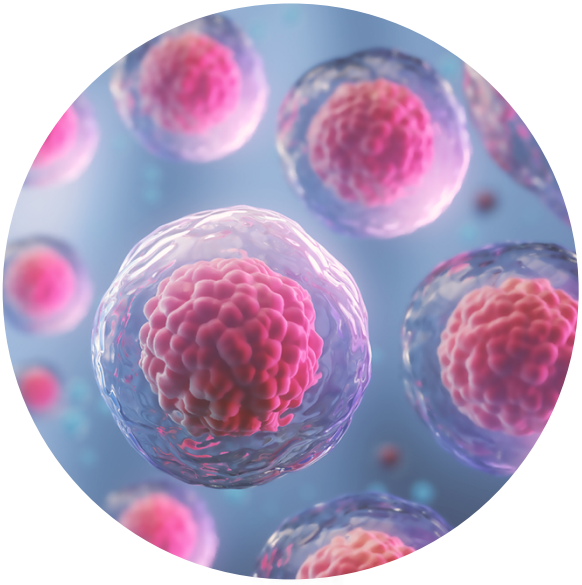
Specification Sheet

Normal Human C14+ Monocytes

HC-0030

This product is for Research Use Only. This product is not approved for human or veterinary use or for use in in vitro diagnostics or clinical procedures.



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Safety and Use Statement

Lifeline recommends storing cryopreserved vials in liquid nitrogen vapor phase. Handle cryopreserved vials with caution. Always wear eye protection and gloves when working with cell cultures. Aseptically vent any nitrogen from cryopreserved vials by carefully loosening the vial cap in a biosafety cabinet prior to thawing the vials in a water bath. If vials must be stored in liquid phase, the vials should be transferred to vapor phase storage or -80°C for up to 24 hours prior to being thawed.

Quick Steps for Thawing Culture:

1. Always wash hands before and after working with cell cultures.
2. Always use aseptic technique when dealing with cell culture.
3. Warm cell culture medium prior to retrieving cells.
4. Aseptically vent any nitrogen from cryopreserved vials in a biosafety cabinet prior to thawing in a water bath.
5. Thaw cells quickly in a water bath until only a small ice chip remains.
6. Clean the vial with Ethanol.
7. Transfer the cells to a conical tube with medium in a sterile biosafety cabinet.
8. Count cells using a hemacytometer.
9. Add cells to culture flasks or multiwell plates.
10. Inoculate cells using 1 mL of culture medium per 5 cm^2 surface area at 37°C, 5% CO_2.

Lifeline Technical Note: There are different and often contradictory terminologies used by cell culture companies to define the passage number of cells. Lifeline's designation of 'primary cells' are cells that have been isolated from tissue, plated onto culture vessels, expanded, harvested and cryopreserved. The term 'secondary' indicates that the cells have been isolated, plated and expanded in culture vessels twice before being harvested for cryopreservation.

Basic Aseptic Technique

Cells, medium and LifeFactors™ should only be used in an aseptic environment, a Class II biological safety cabinet with front access and filtered laminar airflow, or an equivalent device. Always wear gloves and eye protection when working with these materials. Wipe or spray medium bottle and LifeFactors with 70% ethanol or isopropanol before opening, especially around the area of the cap. Allow these surfaces to dry completely before opening the bottle or vials. Transfer cells, medium or LifeFactors with disposable sterile pipettes. Do not mouth pipette! Take up the volume needed into the pipette, being careful not to touch the sterile tip to the rim of the container or any other surface. Close the co again being careful not to touch any surfaces with the sterile tip, again

being careful not to touch any surfaces with the sterile tip. Transfer the material and close the container. Wash your hands before and after working with cell cultures. Do not block airflow in a laminar flow hood as this may compromise sterility. Ensure that biological safety cabinets are certified routinely and that the HEPA filters are replaced regularly.

Medium Preparation

RPMI 1640 (LM-0025) contains no growth factors or antimicrobials. To support mononuclear cell viability and function you must add 10% FBS to the basal medium (see RPMI-1640 Medium instructions). Antimicrobials are not required for mononuclear cell culture. A 1.0 mL vial of Gentamicin and Amphotericin B (GA; LS-1117) is provided with the purchase of LL-0066 RPMI 1640 Complete Kit for your convenience.

All procedures should be executed using aseptic technique (see section on basic aseptic technique). Fetal Bovine Serum LifeFactor (FBS; LS-1012) is sufficient to supplement one 100 mL bottle of RPMI 1640 Basal Medium. RPMI 1640 Basal medium and FBS LifeFactor should be thawed and mixed immediately prior to supplementation. Antimicrobials are not required for mononuclear cell culture but may be added at this step by adding 1.0 mL of Gentamicin and Amphotericin (GA LS-1117). Mix supplemented medium by gently pipetting up and down with a large volume pipette (25 or 50 mL) or gently invert the tightly closed 100 mL bottle. Do not shake or froth the medium. The supplemented medium may be stored at 2 to 8°C for up to two weeks.

Pre-warming Medium

If using less than 100 mL of supplemented (complete) medium, Lifeline recommends warming only the volume needed in a sterile conical tube. Repeated warming of the entire bottle over extended periods may cause degradation of the medium and reduced shelf life. Medium will warm to 37°C in 10-30 minutes, depending on the volume. Do not leave medium in water bath for extended periods.

Cryopreserved Vials

Human CD14+ Monocytes cells are sold as cryopreserved vials and are shipped in insulated packages containing dry ice to ensure the cells remain in a cryopreserved state. To maintain the cells' integrity, unpack the products immediately upon receipt and store at a temperature lower than -150°C or in the vapor phase of a liquid nitrogen dewar. If the cells are to be thawed and plated within 24 hours, they may be stored at -80°C. Do not store the vial for more than 24 hours at -80°C as the cells will slowly degrade at this temperature. PBMC have a short lifespan in culture. RPMI 1640 with 10% FBS will maintain the cells but cytokines specific to the application can be added.

Thawing Cryopreserved Cells

Remove vial from dewar and check the cap to be sure that the vial is securely sealed. Spray the vial with 70% ethanol or isopropanol and transfer it to a biosafety cabinet. Allow it to dry thoroughly and carefully loosen the cap to vent any liquid nitrogen that may have entered the vial. Recap the vial and submerge only the bottom half of the vial in a 37°C water bath for approximately one minute or until vial is almost completely thawed—a small amount of ice should still be visible—thawing past this point may damage the cells. To avoid potential contamination, do not allow the vial cap to make contact with the water. Dry the vial, spray the exterior of the vial with 70% ethanol or isopropanol and place the vial in a biological safety cabinet and allow it to dry. Carefully remove the cap to avoid contamination or spatter. Gently resuspend the cells in the vial using a 1- or 2-mL sterile pipette. Transfer the cells to a centrifuge tube containing 9 mL of pre-warmed medium and mix by gently inverting the tube 2 or 3 times. Remove an aliquot to check the cell count (See "Standard Calculation for Counting of Cells" below.) Because of the cryopreservative used, the remaining cells must be centrifuged for 5 minutes at 200 xg**. Aspirate supernatant and resuspend the cell pellet gently in pre-warmed culture medium to desired concentration. Plate the cells into pre-warmed culture medium in the desired culture vessel. (Please see the Standard Calculation section below.) Gently rock the culture vessel from side to side and front to back to evenly distribute cells within the vessel. Place seeded culture vessel in a 37°C, 5% CO_2 incubator.

****To calculate RPM given the RCF or g, use the following formula**

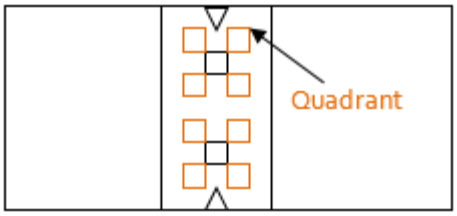
RCF = 0.00001118 × (rpm)² × r or RPM = $\sqrt{RCF/(RPM \times (1.118 \times 10^{-5}))}$

r = rotational radius in centimeters
RPM = rotations or revolutions per minute
RCF Relative Centrifugal Force = g

Standard Calculation for Counting of Cells

Gently re-suspend the cells evenly in the cryovial suspension, or medium if the cells are being passaged. Using a clean hemacytometer and sterile technique, dilute a small volume of cells in trypan blue at a final ratio of 1:100 for HC-0025 and 1:200 for HC-0024. Place 10 µL of the cell suspension into one chamber of the hemacytometer. Count a minimum of 4 quadrants on the hemacytometer (see diagram below). Dead and dying cells are permeable to Trypan Blue, viable cells will not be blue. For accurate cell counts, the optimal number of cells per quadrant should be 25-75 cells. After counting the cells, calculate the average of the 4 quadrants. Take the cell count average and multiply by any dilution factor and by 10^4 to get the number of cells per mL. Multiply the desired seeding density by the surface area of the vessel(s) to be inoculated. This will give you the total number of cells to inoculate one vessel. Divide the number of cells needed to inoculate the vessel(s) by the total number of cells in the cell suspension. This will give you the volume of cell suspension with which to inoculate the vessel(s). Inoculate the cells into the culture vessel(s) prepared with pre-warmed culture medium. Mix gently to evenly distribute the cells and place culture vessel(s) into the incubator at 37°C, 5% CO_2.

Sample calculation:
Average viable cells per quadrant = 31
31 cells/quadrant x 10,000 quadrants/mL x 100 (dilution factor*) = 31,000,000 cells/mL
***If using 1:100 dilution of Trypan Blue and cell suspension.**



Recommended Feeding Guidelines

Guidelines for a T-25 Flask. Adjust volumes according to culture surface area.
Every other day, remove medium and feed with 5 mL of fresh supplemented medium.
Most cultures which are 50% confluent will be ready for passage within two days and should be fed with 7 to 8 mL of supplemented medium.
Do not use more than 10 mL of medium per 25 cm^2 of culture surface to ensure that the media depth is at a level where gas diffusion will be sufficient to support the cells' requirements for oxygen.

The depth of the medium affects gas diffusion gradients through the culture medium to the cells. The volumes of medium recommended in this table result in a range of depths between 2 mm and 5 mm, which is compatible with general recommendations, 10 mL being at the maximum depth allowable (5 mm).

Description	Part #
Human CD14+ Monocytes – 5 x 10^6 cells per vial	HC-0030
RPMI 1640 Medium Complete Kit (RPMI 1640 Basal Medium, Fetal Bovine Serum, Gentamicin and Amphotericin B)	LL-0066
RPMI 1640 Basal Medium, 100 mL	LM-0025
Fetal Bovine Serum, LS-1012, 10 mL	LS-1012
GA Antimicrobial Supplement, 1.0 mL (Gentamicin 3.0 mg/mL, Amphotericin B 1.5 µg/mL)	LS-1117

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