Lonza Walkersville, Inc.
www.lonza.com
scientific.support@lonza.com
Scientific Support: 800-521-0390
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Walkersville, MD 21793-0127 USA

Poietics® Human Preadipocyte Cryoplate

Instructions for Use

Introduction

Poietics® Primary Human Subcutaneous Preadipocytes are isolated from subcutaneous adipose tissue by enzymatic digestion and selective culturing techniques. 96-well culture plates are seeded at 10,000 cells per well with pre-qualified passage 1 cryopreserved Poietics® Primary Human Subcutaneous Preadipocytes. The cells are allowed to attach and then cryopreserved with an overlay of a proprietary cryoprotective agent.

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, 1999. 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. Remove the Preadipocyte Cryoplate package from the dry ice packaging and immediately place into -80℃ freezer for storage. (The Cryoplates may be stored in a -80℃ freezer for up to 1 month from date of shipment). Alternatively, use the Cryoplate immediately (see thawing and recovery instructions below). If no dry ice remains, please contact Customer Service.
3. BulletKit® Instructions: Upon arrival, store basal medium at 4-8℃ and SingleQuots® at -20℃ in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 4℃ and added to basal medium within 72 hours of receipt. After SingleQuots® are added to basal medium, use within one month. Do not re-freeze.

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

Preparation of Medium

1. Use pre-warmed (37°C), supplemented medium for thawing the Cryoplate and culturing preadipocytes.
2. Thaw the FBS, L-Glutamine and the GA-1000 components of the SingleQuots® Kit. (Set aside the other components to make up the Adipocyte Differentiation Medium below).
3. Decontaminate the external surfaces of a 500 ml bottle of Preadipocyte Basal Medium-2 with 70% v/v ethanol or isopropanol. Place the bottle in a biosafety cabinet.
4. Make up Preadipocyte Growth Medium-2 by adding the entire contents of the FBS, L-glutamine, GA-1000 SingleQuots® to the bottle of Preadipocyte Basal Medium-2. Reserve 100 ml of Preadipocyte Growth Medium-2 for subsequent preparation of differentiation medium with the remaining SingleQuots®.

Thawing of Preadipocyte Cryoplate / Initiation of Culture Process

NOTE: The thawing procedure described below has been developed to provide optimal recovery and subsequent cell differentiation. Failure to follow this protocol will result in lower than optimal results.

1. Prepare and pre-warm the supplemented medium as described above.
2. Remove the Cryoplate from the zip style foil bag and the plate sleeve.
3. Immediately place the Cryoplate in a biosafety cabinet and using a multi-channel pipette, add 200 µl of pre-warm supplemented medium to all 96 wells of the frozen plate. **Do not allow the plate to thaw at room temperature, or thaw without medium in the wells.**
4. Place the plate in a 37°C humidified incubator with 5% CO_2 for 2 to 2½ hours to allow the culture to thaw.

a. Remove the culture plate from the incubator and remove the medium (with the cryoprotective agent) from the wells. To avoid disturbing the cell monolayers, it is recommended that the medium be removed by inverting the plate and dumping the medium into a sterile waste container and tapping the plate on a sterile absorbent pad to remove the excess liquid. If an aspirating system is used, care should be exercised not to touch the cell monolayers.

5. Replace the medium in the wells with 100 µl of fresh pre-warmed supplemented medium and return the plate to incubator for 16-24 hours.
6. The culture plate is ready to use for adipogenic differentiation or other assays after the overnight incubation.

Maintenance / Differentiation

To Prepare Adipocyte Differentiation Medium

1. Add the entire contents of the SingleQuots® Vials of insulin, dexamethasone, indomethacin and isobutyl-methylxanthine (IBMX) to 100 ml of Preadipocyte Growth Medium-2 pre-warmed to 37°C.

NOTE: The 100 ml of Differentiation Medium will be "2X". The concentrations of the differentiation agents will be diluted 2-fold when added to the pre-plated cells.

Differentiation of cells

1. Induce the preadipocytes to begin differentiating into adipocytes with the addition of 0.1 ml of Adipocyte Differentiation Medium to each well.
2. If the cells are to be treated with a series of test samples, set up several 24-well dilution plates with the appropriate volume of Preadipocyte Differentiation Medium per well and make the required serial dilutions of the test samples. Add 0.1 ml of each different concentration of test samples to wells of the Preadipocyte Cryoplate. It is recommended that each assay is done in triplicate.
3. We suggest that "control" wells be set up which contain 1) 100 µl of Preadipocyte Growth Medium-2 instead of Differentiation Medium, 2) no added test sample and 3) "solvent only" if the test samples were dissolved in solvents such as DMSO, ethanol, etc.
4. No further additions or medium changes are required. Differentiated adipocytes are delicate and care should be used to avoid disrupting the numerous lipid vacuoles in the cells.
5. The extent of adipocyte differentiation may be noted by microscopic observation of lipid vacuoles in the induced cells. The intracellular lipid vacuoles will begin to appear 4 to 5 days after induction and will continue to increase in number and size for 7 to 10 days. Non-induced cells will have few, if any, lipid vacuoles.
6. To document adipocyte differentiation, cultures may be carefully rinsed with PBS and stained. Poietics® AdipoRed™ Reagent (PT-7009) is a recommended and convenient reagent for the high-throughput assay of *in vitro* preadipocyte differentiation. Alternatively, adipocyte differentiation can be measured by immunoassays or mRNA amplification assays to quantify the expression of proteins such as leptin, AP2 or PPARγ that have been used as "markers" of the differentiated adipocytes.

Ordering Information

Cryopreserved Cells

00190953	Preadipocyte Cryoplate (Subcutaneous)	1 x 96-well plate

Related Products

Preadipocyte Growth Media (Must be purchased separately)

PT-8002	PGM™-2 BulletKit®	Kit which contains a 500 ml bottle of PBM-2, (PT-8202) and PGM™-2 SingleQuots® (PT-9502).
PT-8202	PBM-2	Preadipocyte Basal Medium-2 (no growth factors) (500 ml)
PT-9502	PGM™-2 SingleQuots®	Supplements and growth factors (FBS, L-glutamine, GA-1000, Insulin, Dexamethasone, Indomethacin, 3-Isobutyl-1-methylxanthine)
PT-7009	AdipoRed™ Test Kit	5 X 4.0 ml
17-512F	DPBS	500 ml

Product Warranty

CULTURES HAVE A FINITE LIFESPAN IN VITRO. Lonza guarantees the performance of its cells only if Poietics® Media and Reagents are used exclusively,

and the recommended protocols are followed. The performance of cells is not guaranteed if any modifications are made to the complete Cell System. Preadipocyte Cryoplate (subcutaneous cells) are assured to be viable and functional when thawed and maintained properly.

Quality Control

For detailed information concerning QC testing, please refer to the Certificate of Analysis.