PRODUCT DATA SHEET

NOTICE: Document includes representative data for a specific Lot of primary breast cancer cells. Multiple donor Lots are available. Please contact AMSBIO for information on current donor Lots.

PRODUCT: Primary human breast cancer cell culture CLTH/BC-12

CATALOG NUMBER: CL 04002-CLTH

SHIPPED IN: Dry ice

STORAGE: Storage temperature: liquid nitrogen vapor phase

Note: For best results begin culture of cells immediately upon receipt. If this is not possible, store at -80ºC up to one month or for long term store in liquid nitrogen vapor phase.

PASSAGE: 2

QUANTITY & CONCENTRATION:

Cells are provided to customers in vials containing ≈1 x 10^6 cells/mL in Freeze Medium (FBS supplemented with 10 % (v/v) DMSO)

BACKGROUND/DESCRIPTION

The Primary human breast cancer cell culture CLTH/BC-12 is gained from human breast carcinoma lobulare infiltrans sample. The cells grow adherent. The BC have been characterized as epithelial cells via morphological observation throughout serial passages and positive staining for pan-Cytokeratin. Delivered cells are also positive for MGB-1 specific for breast cells. The BC cells express an isoform 7 of MUC-1 specific for tumor cells.

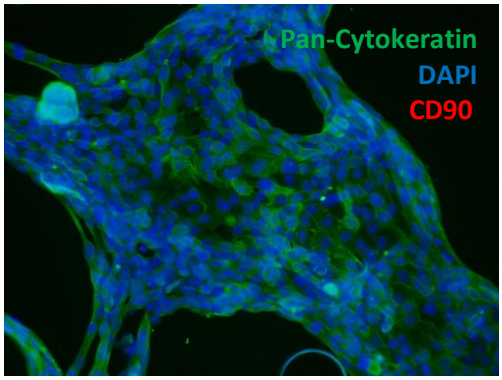

Fig. 1. Immunofluorescence staining using pan-Cytokeratin antibody for detecting epithelial cells. Magnification 10 x 10 microscope. Magnification 10 x 4. No visible fibroblasts fraction (CD90 – red)

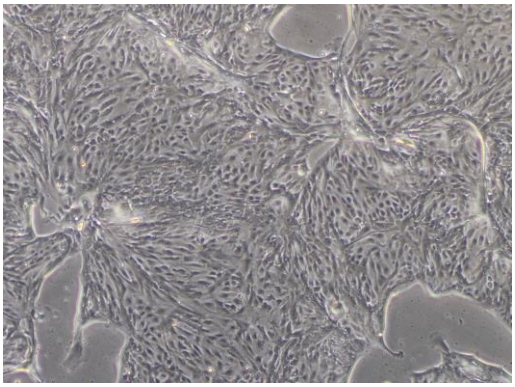

Fig. 1. Phase-contrast microscopy of primary breast cancer cells (magnification 10 x 4)

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CLINICAL DATA

Age: 58
Breast cancer, Infiltrating ductal carcinoma, pT2N2; ER-TS 8/8 (+); PR-TS 5/8 (+); HER 2: 1+ (-); Ki67-10%

QUALITY CONTROL

This cryovial contains at least 1.0 x 10^6. CLTH/BC-12 cells as determined by viable cell count. The CLTH/BC-12 are free of microbial contamination.

MEDIUM

As culture medium we recommend EpiCult™-C Basal Medium (Human) (Catalog #05631) together with EpiCult™-C Proliferation Supplement (Human), (Catalog #05632). Add antibiotics, if desired. Adding of Y-27632 or another ROCK inhibitor to medium can improve culturing results (e.g. 5 to 10 µmol/L of Y-27632 from Stemcell technologies, Cat no.: 72302).

UNPACKING & STORAGE INSTRUCTIONS

1. Check all containers for leakage or breakage.
2. Thaw the frozen cryovial according to subculturing procedure.
3. Optimally: Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below 80°C, preferably in liquid nitrogen vapor, until ready for use.

HANDLING PROCEDURE FOR FROZEN CELLS

Establishing the CLTH/BC-12 cell cultures:

1. The recommended seeding density is 100-200 cells/mm^2.
2. Before thawing cells calculate the number of needed vessels covered with attachment surface such as Collagen Bovine etc. Allow them to equilibrate with desired medium in 37°C, 5% CO_2, 5% O_2 humidified incubator for at least 30 minutes.
   Using dishes coated with collagen or other attachment factors enhances cells survival
3. Place 10 mL of medium in a 15-mL conical tube.
4. Quickly thaw the frozen cryovial in a 37°C water bath. Decontaminate the cryovial by wiping the surface of the vial with 70% (v/v) ethanol.
5. Transfer the cells to the conical tube containing the medium.
6. Centrifugation at 150 x g for 7 minutes at room temperature and then remove the medium.
7. Resuspend the cells in the small amount of fresh medium and transfer to an equilibrated culture dish with desired growth medium.
8. Place the cells in a 37°C incubator at 5% CO_2 and 5% O_2. Monitor the cell density every

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second day.

SUBCULTURING PROCEDURE

1. Discard culture medium.
2. Briefly rinse the cell layer with HBSS and discard it.
3. Add Trypsin-EDTA 0,25% solution to culture dish and place it at 37°C humidified incubator for 3-4 min.
4. Add trypsin neutralizing solution or medium containing serum and scratch cells with a cell scraper.
5. Centrifuge cells 150 x g for 7 min and resuspend cells in fresh growth medium.
6. Add appropriate aliquots of the cell suspension to new culture vessels.
7. Incubate cultures at 37°C, 5% CO_2, 5%O_2

Subcultivation Ratio: A subcultivation ratio of 1:3 is recommended.
Medium Renewal: Thrice per week.

SAFETY PRECAUTION

AMS Biotechnology Europe recommends using protective gloves and clothing and wearing a full face mask always when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. During thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.
The product should be handled by trained personnel observing good laboratory practices. It is important to avoid breathing vapor, avoid skin contact or swallowing.

BIOSAFETY LEVEL: 1

Appropriate safety procedures should always be used with this material. Please check all safety procedures required in your country.

WASTE DISPOSAL

It is highly recommended that waste always be returned to special company responsible for utilizing such type of waste.

WARRANTY

The viability of all cell based products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. Celther Polska outlines the list of media formulation that has been found to be effective for this strain. While other, unspecified media may also give satisfactory results, a change in media or the absence of an additive from the Celther recommended media may cause problems with recovery, growth and/or function of this strain. If an alternative medium formulation is used, the

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