

Depletion of benign cells from established cancer cell cultures with the PromoCell Primary Cancer Culture System D-ACF



Application Note

PromoCell Primary Cancer Culture System D-ACF

Traditional culture systems for cancer cells all share a lack of specificity for malignant cells. Most of the media used predominately support the proliferation of benign cells, e.g. stromal cells, or differentiated (non-tumorigenic) cancer cells, which frequently leads to stromal overgrowth (see Fig. 1) and gradual loss of the malignant cells contained in the original tumor.

The advanced PromoCell culture system, consisting of the Primary Cancer Cell Medium D-ACF and the NCCD-Reagent, was designed to be the first universally applicable, cost-effective solution for *in vitro* isolation of long-term primary cultures of human malignancies, e.g. from patient tumor samples or patient-derived xenografts (PDX).

The Primary Cancer Culture System is a reliable tool for depleting stromal cells, fibroblasts and all other types of non-cancerous cells in any type of established cell culture, regardless whether

a common cancer cell line or a specific primary culture of a human malignancy established with your own protocol is chosen as the source of the true cancer cells. The specificity of the PromoCell Primary Cancer Culture System for malignant cells permits precise control and depletion of unwanted noncancerous cells. Provisional enrichment techniques, e.g. cell sorting while relying on unproven markers, are now obsolete for researchers aiming to obtain the cancer cells they want to study.

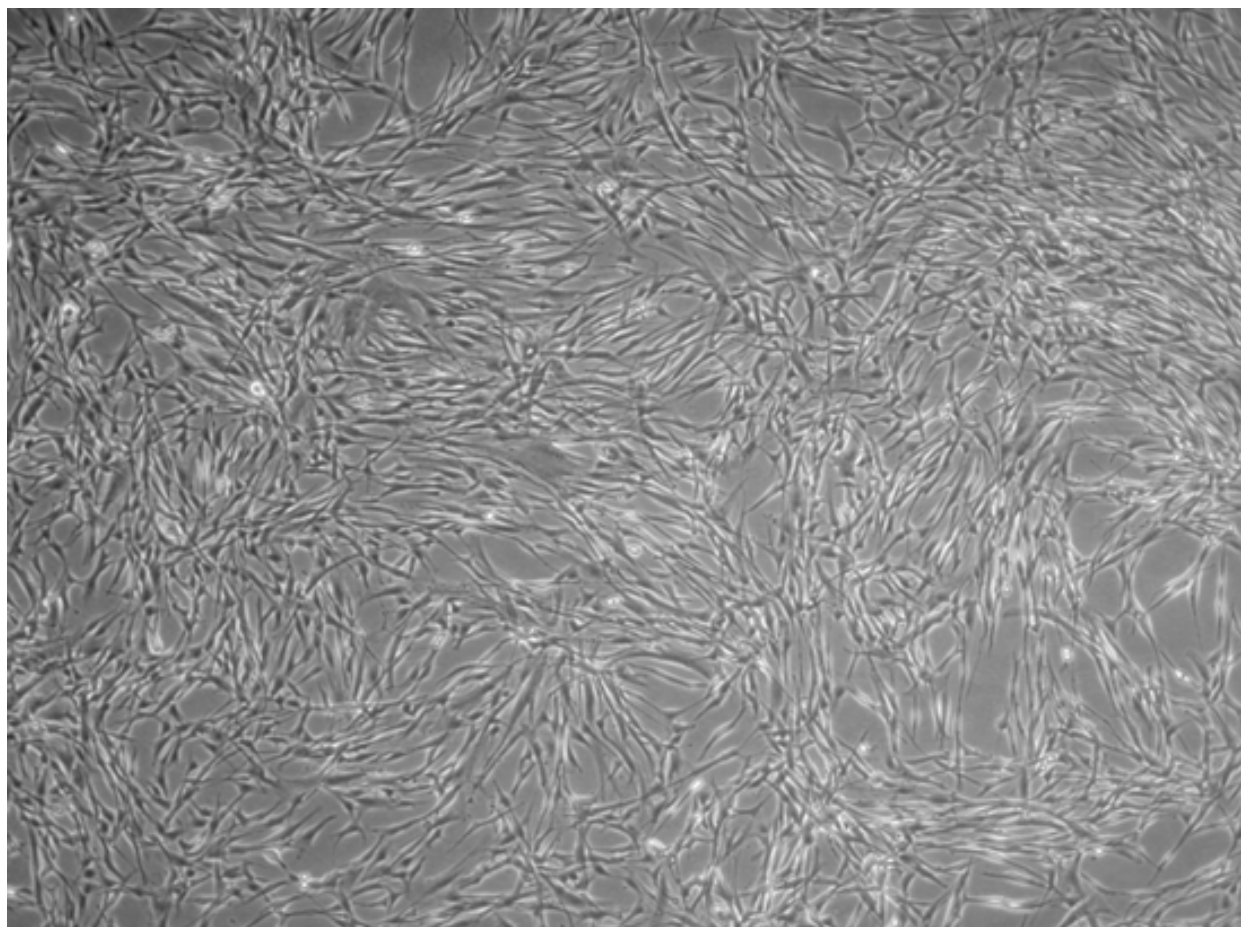


Fig. 1: If your cancer cell cultures often look like this, it may be time to try the PromoCell Primary Cancer Culture System D-ACF. This is a typical stromal overgrowth pattern in a primary osteosarcoma culture at three weeks, using a conventional tumor cell medium. The malignant cells have already been lost, with only fibroblastoid stromal cells remaining.

Use aseptic techniques and a laminar flow bench.

A) Noncancerous Cell Depletion

I. Materials

- Existing culture containing malignant cells, e.g. cell line or primary isolate
- Primary Cancer Culture System (C-28081)*
*consists of the Primary Cancer Cell Medium D-ACF and 2 ml of NCCD Reagent (C-43080; also available separately)
- Phosphate-buffered saline (PBS) w/o $\text{Ca}^{2+}/\text{Mg}^{2+}$ (C-40232)
- Accutase (C-41310)
- Tissue culture treated cell culture vessel

II. Depletion Protocol

1. NCCD treatment of plasticware with TC surface

Dilute the thawed NCCD Reagent stock solution 1:20 with PBS w/o $\text{Ca}^{2+}/\text{Mg}^{2+}$. Treat the tissue culture vessel with 100 μl of diluted NCCD Reagent per cm^2 of the culture surface area and leave the vessel closed for at least 1 hour at RT. Make sure that the NCCD solution covers the entire vessel surface. Aspirate the NCCD solution just before seeding the cells.

Note: Unless the sealed vessel containing the NCCD Reagent will be used right away, it can be stored for up to 3 months at 2 – 8°C for later use. Diluted NCCD solution may be stored for up to 4 weeks at 2 – 8°C if it is protected from exposure to light.

2. Determine the growth pattern of the malignant cells

Passage your established culture containing the malignant cells as usual. Plate a sample of the cells in a NCCD-treated vessel containing an appropriate amount of Primary Cancer Cell Medium D-ACF. Change the medium at least once every 10 days. If the medium turns yellowish-orange before that, just add some fresh medium.

Note: The malignant cells may grow adherently and/or as spheres in suspension. During the induction phase, they may proliferate more slowly than under your established standard culture conditions. However, the culture will recover as soon as the nonmalignant cells have been substantially depleted (in passages 2 and 3) and the cultured cells have fully adapted to the new conditions.

3. Clean up your culture

After identifying the growth pattern of the malignant cells of interest under these culture conditions, passage the culture into Primary Cancer Cell Medium D-ACF. Expand and passage the cells 2 to 3 times as required to clear the culture of nonmalignant cells.

Non-Cancerous Cell Depletion Protocol

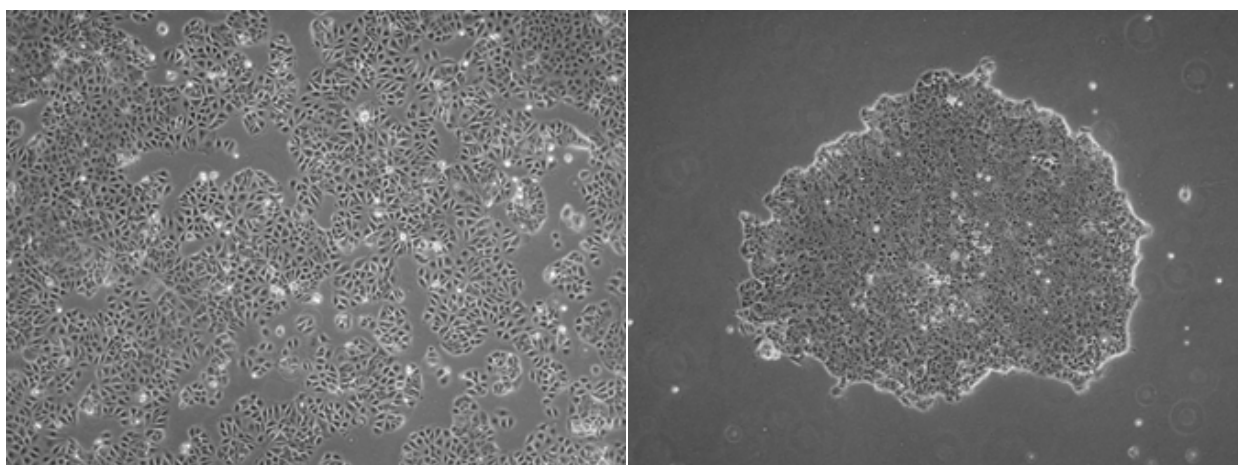


Fig. 2: Morphology comparison of the breast carcinoma cell line MCF-7 before (left) and after (right) 6 weeks of culture with the Primary Cancer Culture System D-ACF. Compared to the original culture in a standard medium (left), the cells selected in the Primary Cancer Culture System D-ACF proliferate more slowly, are decreased in cell size and exhibit an altered growth pattern with compact colonies of cells with a remarkably homogenous appearance.

Background

Tumors consist of a heterogeneous mix of multiple interacting cell types organized in a complex hierarchy. Only a

small subpopulation of the tumor cells are cancer cells capable of driving the progression and, ultimately, dissemination of the malignancy. Most tumors largely consist of non-tumorigenic, dif-

ferentiated cells and benign cancer-associated cells such as cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs) and stromal cells.

Products

Product	Size	Catalog Number
Primary Cancer Culture System consists of		C-28081
Primary Cancer Cell Medium D-ACF	250 ml	C-28080*
Primary Cancer Cell Medium D-ACF SupplementMix	for 250 ml	C-39880*
NCCD-Reagent	2 ml	C-43080

*not available as single item

Related Products

Product	Size	Catalog Number
Dulbecco's PBS, w/o Ca ⁺⁺ /Mg ⁺⁺	500 ml	C-40232
Accutase Solution	100 ml	C-41310
NCCD-Reagent	2 ml	C-43080
Cryo-SFM	30 ml	C-29910
3D Tumorsphere Medium XF	250 ml	C-28070
Cancer Cell Line Medium XF	250 ml	C-28077

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