Cancer stem cell selection from cancer cultures

PromoCell®

Application note

Background

In this application note we describe a method to select cancer stem cells (CSCs) from already established cancer cell lines using our Primary Cancer Culture System (PCCS).

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 stem cells, capable of driving the progression and, ultimately, dissemination of the malignancy. Most tumors largely consist of non-tumorigenic, differentiated cells and benign cancer-associated cells such as cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs) and stromal cells.

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.

As part of our cancer media toolbox, the Primary Cancer Culture System (PCCS), 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 and long-term cultures of human cancer stem cells (CSCs), e.g. from biopsies or patient-derived xenografts (PDX).

Due to the specific support of the aberrant metabolic traits of malignant cells, the PCCS 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 you work with a common cancer cell line or a specific primary culture of a human malignancy established with your own protocol. The specificity of the PCCS for CSCs permits precise control and depletion of unwanted non-cancerous cells. This makes provisional enrichment techniques, e.g. cell sorting while relying on unproven markers, obsolete.

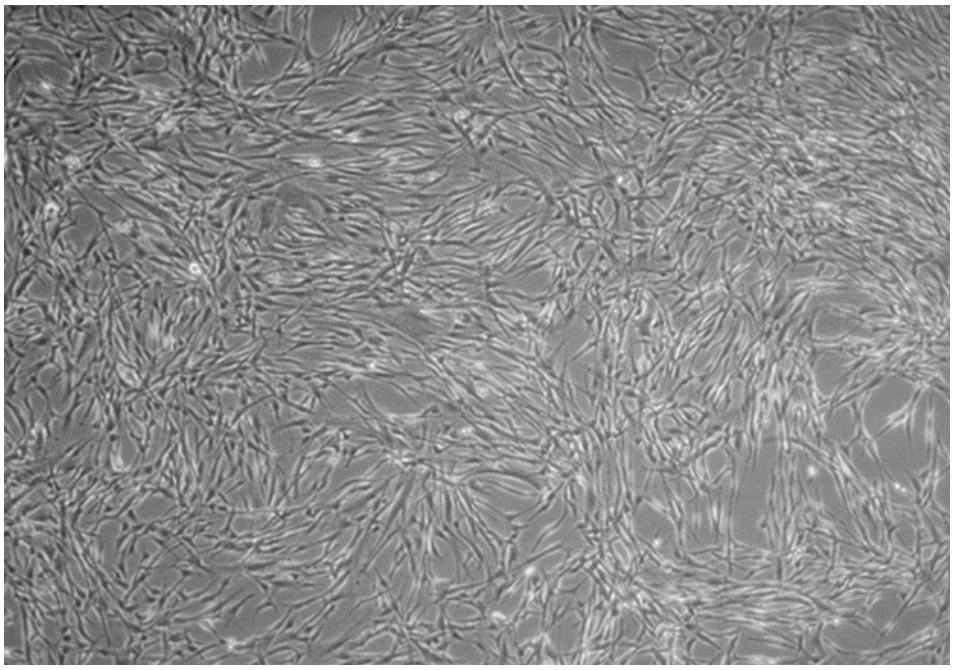


Fig. 1: If your cancer cell cultures often look like this, it may be time to try our Primary Cancer Culture System (PCCS). *This is a typical stromal overgrowth pattern in a primary osteosarcoma culture at three weeks, using a conventional tumor cell medium. The malignant cells have already been lost,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

Protocol for stem cell selection from cancer cultures

I. Depletion of non-cancerous cells

Materials

- Existing culture containing cancer stem cells, e.g. cell line or primary isolate
- Primary Cancer Culture System (PCCS, 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) without Ca^{2+}/Mg^{2+} (C-40232)
- Accutase (C-41310)
- Tissue culture treated cell culture vessel

Use aseptic techniques and a laminar flow bench.

1

NCCD treatment of plasticware with TC surface

Dilute the thawed NCCD Reagent stock solution 1:20 with PBS without Ca^{2+}/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 one hour at room temperature. 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

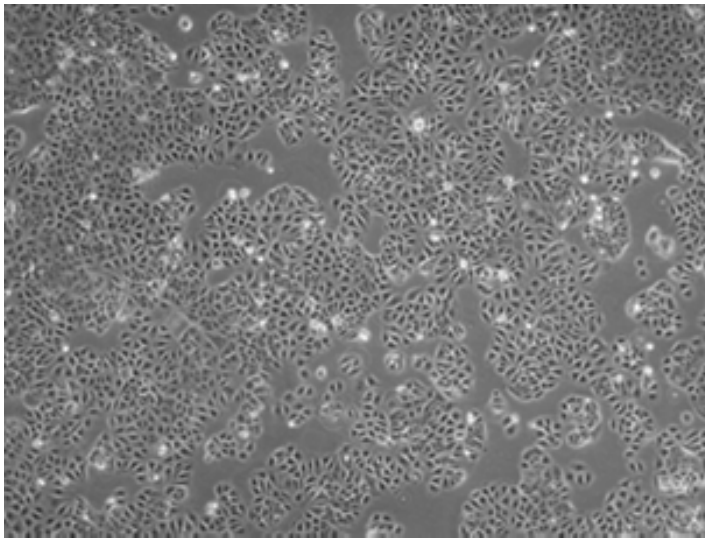
Passage your established culture containing the malignant cells as usual. Plate a sample of the cells in a NCCD-treated vessel containing an appropriate amount of Primary Cancer Cell Medium D-ACF. Change the medium at least once every 10 days. If the medium turns yellowish-orange before that, just add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

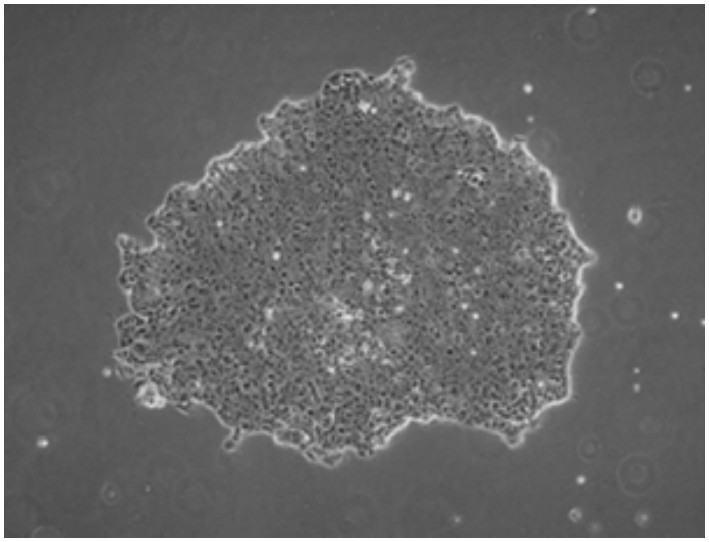
Note: The malignant cells may grow adherently and/or as spheres in suspension. During the induction phase, they may proliferate more slowly than under your established standard culture conditions. However, the culture will recover as soon as the non-malignant cells have been substantially depleted (in 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 2–3 times as required to clear the culture of non-malignant cells.





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 (PCCS). *Compared to the original culture in a standard medium (left), the cells selected in the PCCS proliferate more slowly, are decreased in cell size and exhibit an CSC-typical altered growth pattern with compact colonies of cells with a remarkably homogenous appearance.*

Products

Media	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
Dulbecco's PBS, without Ca^{++}/Mg^{++}	500 ml	C-40232
Accutase Solution	100 ml	C-41310

Related Products

Media	Size	Catalog number
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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