Establishment of non-selective adherent primary cultures from dissociated samples of solid human tumors and xenografts

PromoCell

Application Note

Serum-free, non-selective primary culture of cells derived from tumor samples

PromoCell Cancer Cell Line Medium XF (C-28077) was originally developed as a "one-for-all" serum- and xeno-free culture medium for standardized routine culturing of established adherently growing cancer cell lines.
This medium's formulation provides broad growth support for a variety of adherently growing cell types. I can therefore also be used for other types of applications, e.g. for co-culturing of cell types / populations isolated from primary tumor samples or tumor xenografts. In the context of propagation of cells derived from tumors, Cancer Cell Line Medium XF is a good choice for short-term co-culturing of mixed cell populations obtained dissociating tumor tissue.

In order to provide optimal customization options, Cancer Cell Line Medium XF does not contain any attachment factors, and the cultureware requires an appropriate coating for effective cell adhesion (see section A)II).

The universal, non-selective growth-promoting properties of Cancer Cell Line Medium XF permit *in vitro* expansion of a wide variety of adherently growing cells contained in the original tumor sample, e.g. cancerous cells, cells of the tumor stroma, and other benign cell types. Consequently, in most cases mixed co-cultures of complete cell entities from the original tumors are obtained.
Depending on the tumor sample used, stromal overgrowth accompanied by loss of the original cancer cells may also occur with prolonged culturing. In contrast to the selective PromoCell Primary Cancer Culture System (C-28081), long-term maintenance and enrichment of cancer cells is like to require additional steps with the non-selective Cancer Cell Line Medium XF.

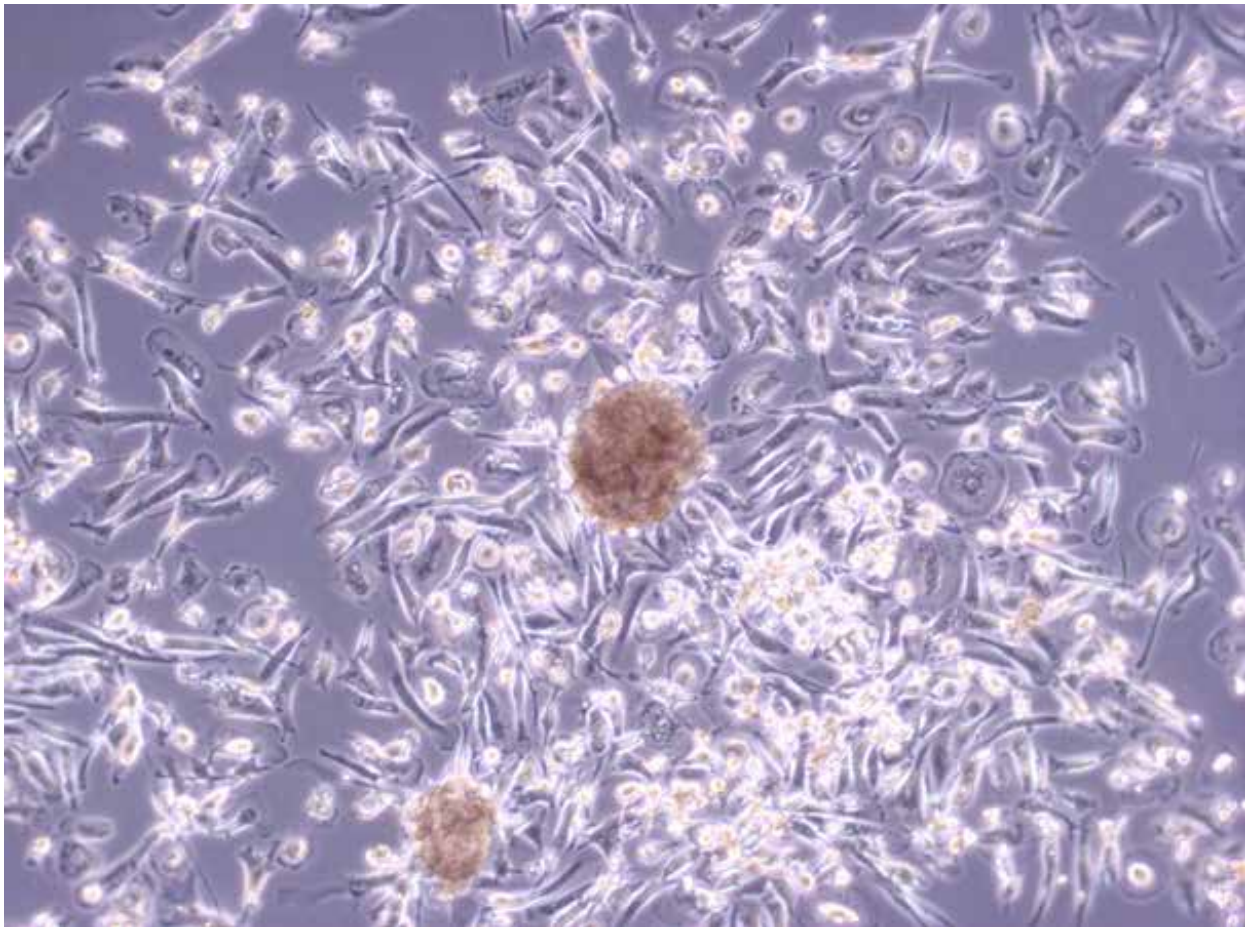

Fig. 1: Day seven mixed primary culture of a lung mesothelioma cultured in Cancer Cell Line Medium XF. The tissue culture vessel was coated with fetal calf serum as an attachment substrate. A mixed population of loosely adherent, brownish cancer cell aggregates and stromal cells of fibroblast- and macrophage-like morphology can be observed (100x magnification).

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A) Protocol for isolating and nonselectively culturing cells from dissociated tumor samples and xenografts

I. Materials

Cancer Cell Culture Materials

- Fresh tumor tissue (0.2 – 3 grams; ≥ 1 gram is optimal)
- Hanks Balanced Salt Solution (HBSS) with Ca^{2+}/Mg^{2+} w/o Phenol Red
- Cancer Cell Line Medium XF (C-28077)
- Gentamicin (50 mg/ml stock)
- Phosphate-buffered saline (PBS) w/o Ca^{2+}/Mg^{2+} (C-40232)
- Accumax (e.g. Sigma #A7098) – tissue digestion/isolation
- Accutase (C-41310) – passage/subcultivation of established culture
- Scalpel/forceps/scissors
- Cell strainers of descending sizes down to 40 µm (e.g. 400/100/40 µm)
- Tilt-roll-shaker, rotary mixer or comparable
- Tissue culture flasks and dishes

II. Preparation of Plasticware (day 0 or earlier)

Cancer Cell Culture Protocol

Coat the plasticware with a suitable attachment substrate according to your standard protocol or according to the instructions of the product's manufacturer.

Note: The choice of a suitable surface coating is up to the customer. As a general rule, it is always a good starting point to use an extracellular matrix coating that is compatible with the corresponding primary cell type (i.e. the kind of tumor cells to be cultured) in serum-free culture.

If you have no information on a suitable attachment substrate or if you want to use a universal matrix for cell adhesion, coating the vessels with FBS (100 µl/cm², 2 hours, RT) may be a viable option.

III. Tumor Cell Isolation Procedure (day 0)

Note: Alternative techniques for dissociation / homogenization of the tumor tissue can also be used. Continue with step 10 when using your own tissue dissociation protocol.

1. **Wash and weigh the tumor tissue**
   Remove any visible remnants of healthy tissue from the tumor. Place the tumor sample in a tube and wash twice with a generous amount of PBS and vigorous shaking. Then weigh the tumor tissue in a pre-tared sterile petri dish.
   Note: The tumor tissue should be as fresh as possible and stored in HBSS at 2 to 8°C immediately after surgical removal.

2. **Homogenize the tumor tissue**
   Place the washed tumor sample on the lid of a petri dish. Add a small volume (1 – 2 ml) of Cancer Cell Line Medium XF to the tumor tissue and dissect it into small pieces using a scalpel. Homogenize the tissue into a "slurry" or make small pieces of approx. 1 mm³ each by additionally mincing the tissue chunks with the scalpel. Avoid attrition of the tissue.

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3. Wash the homogenized tumor tissue

Transfer the homogenized tumor tissue to a 50 ml tube using forceps. Add 10x the volume (w/v) of PBS and vortex or mix vigorously. Let the tissue pieces settle for 2 minutes and then aspirate the supernatant. Repeat if there considerable blood and/or debris are still visible. Aspirate as much as possible of the PBS without losing the tissue.

Note: If there is floating homogenized tissue, use a sieve, e.g. 400 µm, for separating the washed, homogenized tissue from the washing buffer.

4. Enzymatically digest the tumor tissue

Resuspend the tissue pellet in Accumax solution with a concentration of 20 ml per gram of tumor tissue. Incubate at RT (20 – 25°C) with gently but constantly mixing, e.g. in a tilt-roll mixer at 50 rpm. Digest until the solution becomes distinctly turbid. Depending on the tissue type, this usually takes about 30 – 60 minutes. A 45-minute incubation is a good starting point.

Note: Do not digest the tissue longer than necessary and never digest for longer than 60 min, since this may significantly compromise cell viability. Always perform the digestion reaction at RT and consult the Accumax manual for instructions on proper storage and handling.

5. Remove tissue residues from the sample

Let the remaining tissue pieces settle for 2 min. In order to obtain a single cell suspension, progressively filter the turbid supernatant using cell strainers of descending pore size down to 40 µm, e.g. 400 µm, 100 µm, 40 µm.

Note: Discard the remaining tissue pieces.

6. Dilute the sample with medium

Dilute the single-cell suspension at least 1:1 with Cancer Cell Line Medium XF. Use a higher dilution ratio if the solution is still viscous.

7. Obtain the isolated single cells

Pellet the cell suspension for 10 min at 240 x g at RT and carefully aspirate the supernatant without disturbing the cell pellet.

8. Determine the number of viable nucleated cells

Resuspend the cell pellet in 5 ml of Cancer Cell Line Medium XF. Determine the number of viable nucleated cells using an appropriate method.

Note: If cell clumps form and cannot be resuspended, filter the cell suspension once more through a 40 µm cell strainer before counting. The expected yield is 1 – 3 million viable nucleated cells per gram of tumor tissue. If, for any reason, it is impossible to determine the viable nucleated cell count in the primary isolate, continue with step 9 and refer to the note in step 10. Keep in mind that unless the cells are counted, suboptimal seeding densities may result.

9. Wash the cells

Pellet the cell suspension for 10 min at 240 x g at RT and carefully aspirate the supernatant without disturbing the cell pellet. Finally, resuspend the cell pellet in 1 ml of Cancer Cell Line Medium XF.

10. Plate the cells

Plate 100,000 to 200,000 viable nucleated cells per cm^2 in tissue culture vessel(s) that are precoated with your choice of attachment substrate. Use ap-

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prox. 200 µl of medium per cm^2 for vessels with ≤ 25 cm^2 of culture surface and approx. 130 µl medium per cm^2 for > 25 cm^2. Add 50 µg/ml of Gentamicin to the final volume and incubate at 37°C with 5% CO_2.

Example: Plate 2.5 – 5 million nucleated viable cells per T-25 flask using 5 ml of medium. Plate 7.5 – 15 million nucleated viable cells per T-75 flask with 10 ml of medium.

Note: If the viable nucleated cell count was not determined in step 8, plate the primary isolate with up to 2 grams of tumor tissue in a T-25 flask using 5 ml of Cancer Cell Line Medium XF.

IV. Culturing the tumor cells

1. **Initiate of the primary tumor cell culture (day 0)**
   Incubate the culture for 1 – 3 days to initiate the primary tumor cell culture.

2. **Perform the first medium change (day 1+)**
   After 1 – 3 days, introduce the cells into fresh Cancer Cell Line Medium XF (w/o antibiotics).

3. **Let the cells grow (day 3+)**
   Perform a medium change every 2 – 3 days until the primary culture reaches 80 – 90% confluency.

   Note: Cancer Cell Line Medium XF provides general non-selective growth support for a variety of different cell types. The obtained primary culture will therefore probably consist of a mixture (co-culture) of different cell types contained in the original tumor, i.e. cancer cells as well as various types of benign cells contained in the tumor stroma. Additional steps may be required in order to propagate, enrich or isolate any malignant cancer cells present in the tumor cell primary culture. Otherwise stromal overgrowth may occur with prolonged culturing.

4. **Use the cells for your experiments**
   The expanded cells may now be used for your experiments, e.g. cell-based assays, marker analysis or enrichment / purification procedures. Optionally, the culture may also be continued (see step 5).

5. **Subculture of the tumor cell culture (optional)**
   Make sure to precoat vessels with an appropriate attachment substrate (see A)II). Perform a 1:1 or 1:2 split of the culture using Accutase (not Accumax). Wash the culture twice with PBS w/o Ca^{2+}/Mg^{2+} at ambient temperature then incubate the cells for 5 – 10 minutes with 150 µl/cm^2 Accutase at 37°C. After the first 5 minutes of incubation, visually check the detachment process. When the cells start to detach, facilitate their complete dislodgement by tapping the flask. Add the same volume of Cancer Cell Line Medium XF to the detached cells and spin down for 5 minutes at 300 x g at RT. Carefully aspirate the supernatant and gently resuspend the cell pellet in Cancer Cell Line Medium XF. Seed the cells into precoated vessels and incubate them further at 37°C and 5% CO_2.

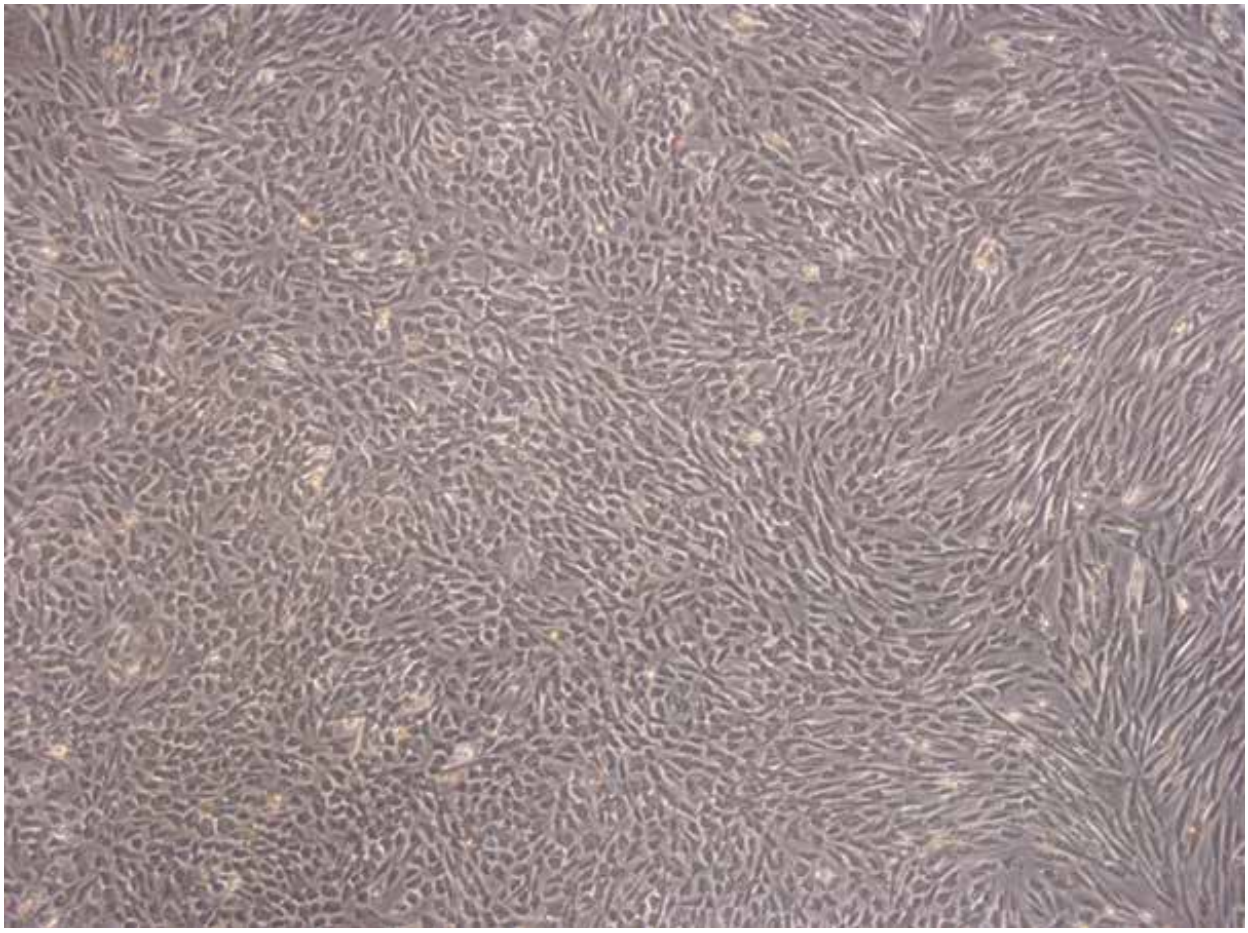

Fig. 2: Mixed culture of cells isolated from an aggressive renal carcinoma (from lung metastatic site) two weeks after seeding. The tissue culture vessel was coated with vitronectin as the attachment substrate. While the epitheloid tumor cells still comprise a major part of the culture (left side of the image), fibroblastoid stromal cells begin proliferating vigorously and then progressively displace the original cancer cells (right side of the image). (40x magnification)

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, the dissemination of the malignancy. The largest share of cells in most tumors consist of non-tumorigenic, differentiated cells and benign cancer-associated cells such as cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs) and stromal cells.

Isolating and culturing cells from dissociated tumors in culture systems to provide general, non-selective growth support results in mixed co-cultures of cancer cells and a variety of benign cell types contained in the tumor stroma.

Non-selective culturing of the overall cell entity of the tumor is a prerequisite for studying cancer cell stroma interactions, e.g. by exploring the stroma composition and its tumor-specific traits. However, due to the lack of selectivity of these culture systems, rapid overgrowth of the cancerous tumor cells by the benign cell fraction is a frequently observed phenomenon.

Products

Product	Size	Catalog Number
Cancer Cell Line Medium XF	250 ml	C-28077
Fibronectin solution, human (1 mg/ml)	5 ml	C-43060
Vitronectin, human, recombinant (HEK)	500 µg	C-69201
Accutase-Solution	100 ml	C-41310

Related Products

Product	Size	Catalog Number
Primary Cancer Culture System	250 ml	C-28081
3D Tumorsphere Medium XF	250 ml	C-28070
Dulbecco's PBS, w/o Ca^{++}/Mg^{++}	500 ml	C-40232
Fibronectin solution, bovine (1 mg/ml)	5 ml	C-43050
DetachKit	3 x 125 ml	C-41210
Cryo-SFM	30 ml / 125 ml	C-29910 / C-29912

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