Standardized Culture of Established Cancer Cell Lines under Serum-free and Xeno-free Conditions

PromoCell

Application Note

PromoCell's Cancer Cell Line Medium XF

Traditionally, established cell lines have been propagated almost exclusively in standard culture media supplemented with significant amounts (2–20%) of fetal calf serum (FCS). Like other undefined media supplements, fetal calf serum has unwanted physiological, genetic and epigenetic cellular effects [1-4] and is known to cause enormous experimental variability and distort readouts and results, e.g. in drug screenings and hormone-related studies [5].

With the aim of achieving a universal, consistent and xeno-free environment for culturing most of the commonly used human cancer cell lines, the PromoCell Cancer Cell Line Medium XF was designed as a defined formulation with the exception of highly purified human plasma-derived albumin. It has no ill-defined components such as fetal calf serum, extracts or hydrolysates and exhibits very low lot-to-lot variability. Since the formulation does not contain any cellular attachment factors, it is necessary to coat culture vessels with fibronectin, vitronectin or other suitable attachment substrates. Being broadly usable across all common adherently growing cancer cell lines, this new culture medium is a cost-effective solution for ensuring efficient, genuinely standardized routine cultures.

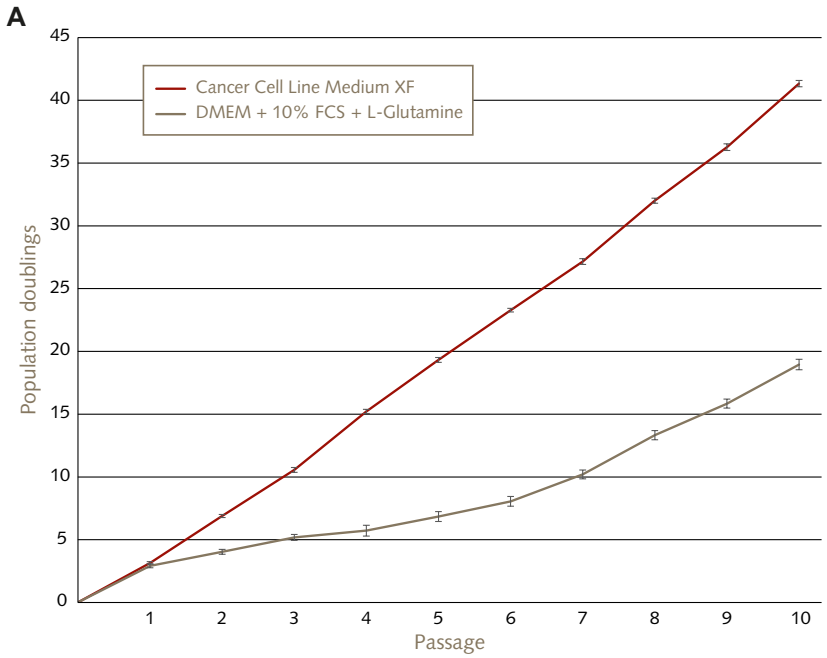
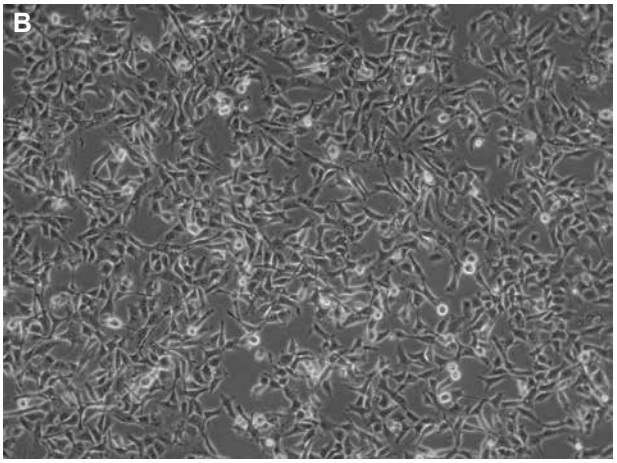
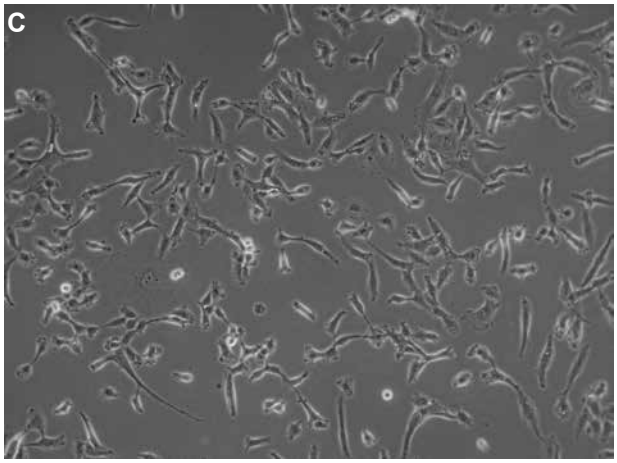


Fig. 1 A: Expansion performance of the HT1080 fibrosarcoma cell line in the Cancer Cell Line Medium XF compared with conventional culture conditions. HT1080 cells were plated with 5,000 cells/cm² in Cancer Cell Line Medium XF on fibronectin-coated vessels (red) or in DMEM + 2 mM L-Glutamine + 10% FCS (grey). Subsequently, the cells were cultured for 10 consecutive passages with a passage interval of 3–4 days.

B, C: Morphology of HT1080 fibrosarcoma cells cultured in Cancer Cell Line Medium XF. Exemplary images of HT1080 on day three after subculture (P7) are shown in the Cancer Cell Line Medium XF (**B**) and conventional culture conditions (**C**) (100x magnification).





Background

For cost reasons, immortal cancer cell lines are widely used in research instead of primary cells. They are accepted, well-defined model systems that serve as a constant source of cells while avoiding the limitations posed by the finite lifespan of normal primary cells [6-9]. As long as the limitations inherent in substituting cell lines for primary cells are taken into account, tumor cells can be used in *in vitro* models to reflect certain aspects and functionalities of terminally differentiated primary human cells [7].

However, poorly defined culture media components such as fetal calf serum are a well-known and significant source of variations [1-5]. These can cancel out the advantages of using cancer cell lines as a uniform source of different types of cells while endangering the reliability of experimental results obtained with them.

As it has been demonstrated for hormone-responsive cell lines, immune-system-related cell types and stem cells, undefined conventional culture media can interfere with cell properties and experimental readouts, for instance by falsifying their responses to drugs, provoking unspecific bogus immune responses or causing unwanted differentiation of stem cells [1, 3, 10]. Cells can also be significantly altered by culture conditions of this kind [11]. It follows that a controlled culture environment is key for obtaining more accurate results with cell line models and facilitates data analysis and interpretation [4], thus taking a major step forward in exploiting the unique features of permanent cell lines as cost-effective *in vitro* research models.

Supplementary Data

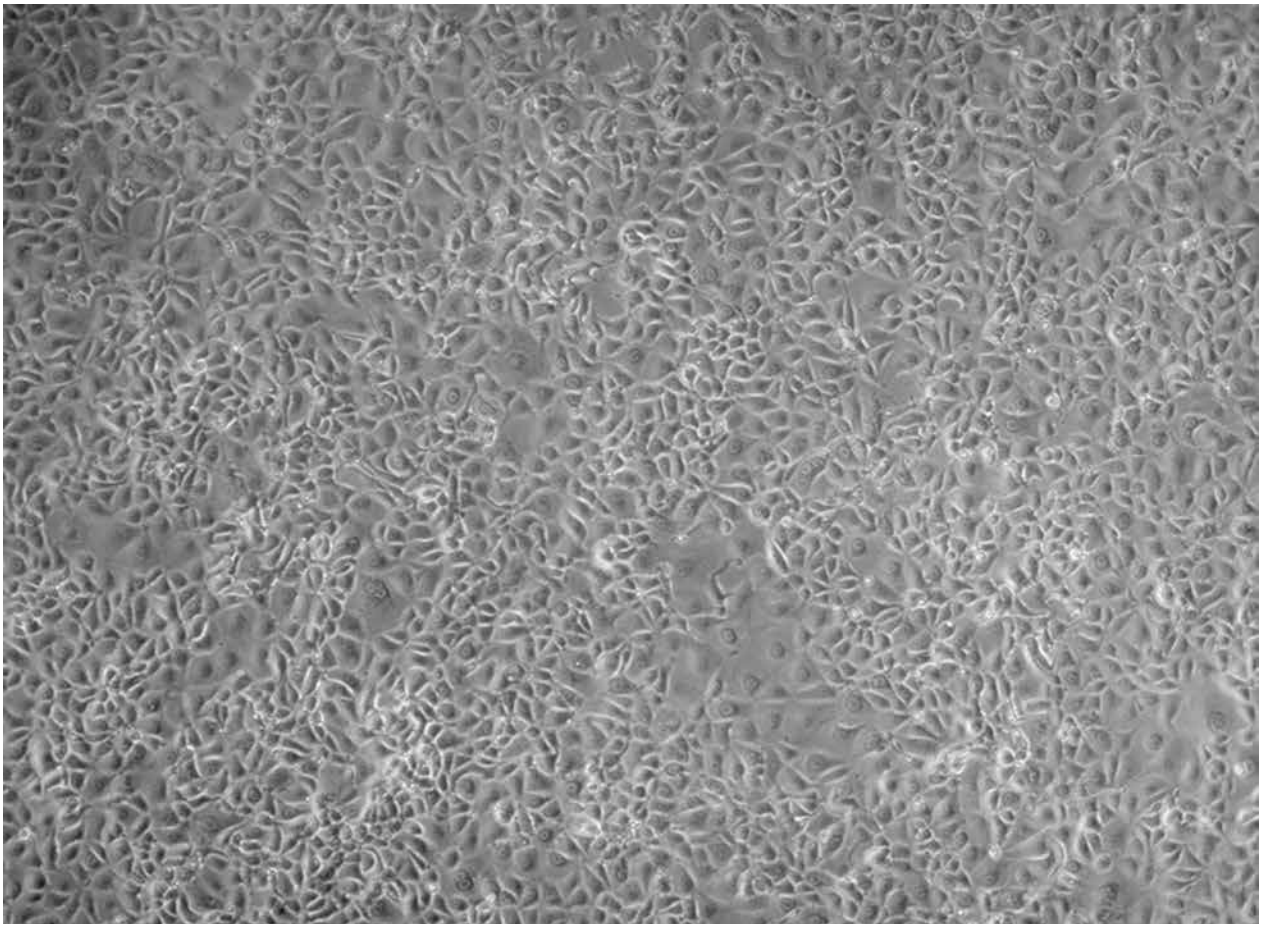


Fig. 3: Morphology of MCF-7 breast carcinoma cells in the Cancer Cell Line Medium XF. MCF-7 cells were plated at 10,000 cells per cm^2 in fibronectin-coated vessels and cultured for three passages in the Cancer Cell Line Medium XF. The cells exhibited efficient proliferation as well as a typical - but slightly more compact - morphology as compared to traditional culture media (not shown). The image was taken 5 days after seeding at 100x magnification.

Use aseptic techniques and a laminar flow bench.

A) Switching an existing cell culture to the Cancer Cell Line Medium XF

This protocol describes how a human cancer cell line can be switched to the Cancer Cell Line Medium XF for the first time.

Cancer Cell Line Culture Materials

I. Materials

- Proliferating culture of a human cancer cell line in good condition
- Cancer Cell Line Medium XF (C-28077)
- Adhesion factors: Human Fibronectin (C-43060) or vitronectin (C-69201)
- Phosphate buffered saline (PBS) w/o Ca^{2+}/Mg^{2+} (C-40232)
- Accutase (C-41310) or, optionally, DetachKit (C-41210)
- Tissue-culture-treated cell culture vessels

Cancer Cell Line Culture Protocol

II. Culture protocol

1. **Coat the culture vessel**
   The serum- and xeno-free formulation does not contain attachment factors. Thus coating of the surface of the cell culture vessel with an appropriate adhesion factor is usually needed. Table 1 (p. 5) shows an overview of cell lines and surface coatings tested with the Cancer Cell Line Medium XF.
   For the establishment of the culture conditions, it is recommended to test fibronectin and vitronectin coating: Coat the culture vessel with 10 µg/ml human (or bovine) fibronectin or 5 µg/ml vitronectin according to the instruction manual of the product. Use 100 µl of diluted coating solution per cm^2 of culture surface. (Final concentration: fibronectin 1 µg/cm^2 and vitronectin 0.5 µg/cm^2)
   Note: If not used immediately, the sealed vessel may be stored for up to 3 months at 2 – 8°C for later use.

2. **Harvest cells from your existing culture**
   Harvest and count cells from an established culture of the appropriate cell line using your standard method. Resuspend them in Cancer Cell Line Medium XF.

3. **Plate the cells**
   Plate the cells at a density of 5,000 – 10,000 cells/cm^2. When seeding the cells for the first time in the Cancer Cell Line Medium XF, use approximately 200 µl of medium per cm^2 of culture surface, e.g. 5 ml for a T25 flask.

4. **Let the cells grow**
   Incubate the plated cells at 37°C and 5% CO_2. Change the medium every 2 – 3 days.
   Note: Adaption of cell cultures to the Cancer Cell Line Medium XF is not required. With some cell lines, proliferation may be somewhat reduced after initiating the culture but this should normalize after one to three passages.

5. **Cell subculture**
   Once the cells have reached 70 – 80% confluence, wash the culture twice with ambient tempered PBS w/o Ca^{2+}/Mg^{2+} and then incubate the cells for 5 – 10 minutes with 150 µl/cm^2 Accutase at 37°C. After the first 5 minutes of incubation, monitor the detachment process visually. When the cells start to detach, facilitate their complete dislodgement by tapping the flask. Add the same

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Cancer Cell Line Culture Protocol

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 an adequate amount of Cancer Cell Line Medium XF. Seed the cells into new fibronectin-coated vessels and incubate them further at 37°C and 5% CO_2. Use approx. 300 – 400 µl of medium per cm^2 of culture surface for the subsequent cultivation. Continue incubation of the cultures at 37°C and 5% CO_2.

B) Routine culture using the Cancer Cell Line Medium XF

This protocol describes the routine culture of a human cancer cell line already transfered to the Cancer Cell Line Medium XF

Cancer Cell Line Culture Materials

I. Materials

See Page 3

II. Culture protocol

1. Coat the culture vessel

Coat the culture vessel with the suitable adhesion factors as previously tested (see page 3).

2a. Harvest cells from your existing culture

Harvest and count cells from an established culture of the appropriate cell line using your standard method. Resuspend them in Cancer Cell Line Medium XF.

Cancer Cell Line Culture Protocol

2b. Thaw cells from cryo-stock

Cryopreserved cells cultured previously in Cancer Cell Line Medium XF may also be thawed directly using this medium.

3. Plate the cells

Plate the cells at the appropriate density (5,000 – 10,000 cells/cm^2). Use approx. 300 – 400 µl of Cancer Cell Line Medium XF per cm^2 of culture surface, e.g. 7.5 – 10 ml for a T25 flask.

4. Let the cells grow

Incubate the plated cells at 37°C and 5% CO_2. Change the medium every 2 – 3 days.

5. Cell subculture

Once the cells have reached 70 - 80% confluence, wash the culture twice with ambient tempered PBS w/o Ca^{2+}/Mg^{2+} and then incubate the cells for 5 – 10 minutes with 150 µl/cm^2 Accutase at 37°C. After the first 5 minutes of incubation, monitor the detachment process visually. 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 an adequate amount of Cancer Cell Line Medium XF. Seed the cells into new coated vessels and incubate them further at 37°C and 5% CO_2. Use approx. 300 – 400 µl of medium per cm^2 of culture surface. Continue incubation of the cultures at 37°C and 5% CO_2.

Table 1: Overview of cell lines and surface coatings tested with the Cancer Cell Line Medium X. Cells were seeded at a density of 10,000 cells/cm².

Tissue	Tested Cell Line	Cell Line Origin	Remarks
Brain	BV2	immortalized murine primary microglial cells	Coat with Fibronectin (C-43060): 1 µg/cm²
Breast	MCF-7	pleural effusion of metastatic human breast adenocarcinoma	Coat with Fibronectin (C-43060): 1 µg/cm²
Colon	HT-29	human colon adenocarcinoma	Coat with Vitronectin (C-69201): 0.5 µg/cm²
Connective tissue	HT 1080	human fibrosarcoma	Coat with Fibronectin (C-43060): 1 µg/cm²
Liver	HepG2	hepatocellular carcinoma of the human liver	Coat with Vitronectin (C-69201): 0.5 µg/cm²
Lung	A-549	human lung carcinoma	Coat with Vitronectin (C-69201): 0.5 µg/cm²
Prostate	LNCaP	lymph node metastasis of human prostate adenocarcinoma	3D culture in C-28070 is recommended

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
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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