Cancer Cell Line Medium XF

PromoCell®

Instruction Manual

Product	Size	Catalog Number
Cancer Cell Line Medium XF	250 ml	C-28077

Recommended for

- Adherently growing established human cancer cell lines

Product Description

The PromoCell Cancer Cell Line Medium XF was designed as a highly standardized serum-free, xeno-free formulation for the serial *in vitro* cultivation of established cancer cell lines. It has no undefined components such as fetal calf serum, extracts or hydrolysates and exhibits very low lot-to-lot variability and requires fibronectin-coated vessels. The Medium consists of a bottle of Basal Medium and one vial of SupplementMix. Adding the SupplementMix to the Basal Medium results in the complete medium.

Note: The serum- and xeno-free formulation means non-availability of attachment factors. Hence, coating with an appropriate adhesion factor is a pre-requisite. For the establishment of the culture conditions, it is recommended to test fibronectin and vitronectin coating. Table 1 (p. 4) shows an overview of cell lines and surface coatings tested with the Cancer Cell Line Medium XF.

Supplementation Details

The PromoCell Cancer Cell Line Medium XF contains all growth factors and supplements. The medium does not contain antibiotics or antimycotics and is formulated for use in an incubator with an atmosphere of 5% CO_2.

Preparation of the supplemented Medium for Use

Thaw the SupplementMix at 15 to 25°C. Aseptically mix the supplement solution by carefully pipetting up and down. Then, transfer the entire content of the SupplementMix to the Basal Medium. Close the bottle and swirl gently until a homogenous mixture is formed.

Storage and Stability

Store the Basal Medium at 2 to 8°C in the dark, store the SupplementMix at -20°C immediately after arrival. Do not freeze the Basal Medium. If stored properly, the products are stable until the expiry date stated on the label. After adding the SupplementMix to the Basal Medium, the shelf life of the complete medium is 6 weeks at 2 to 8°C. For use, pre-warm only an aliquot of the complete medium and keep the remaining medium refrigerated at 2 to 8°C.

Quality Control

All lots of the PromoCell Cancer Cell Line Medium XF are subjected to comprehensive quality control tests using a human cancer cell line. Each lot of PromoCell Cancer Cell Line Medium XF is tested for the ability to support proliferation and serial passage of MCF-7 breast carcinoma cells on fibronectin-coated dishes. In addition, all lots of media have been tested for the absence of microbial contaminants (fungi, bacteria, mycoplasma).

Intended Use

The products are for *in vitro* use only and not for diagnostic or therapeutic procedures. For safety precautions please see appropriate MSDS.

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

Cancer Cell Line Culture Materials

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

I. Materials

See Page 3

II. Culture protocol

Cancer Cell Line 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.

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

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