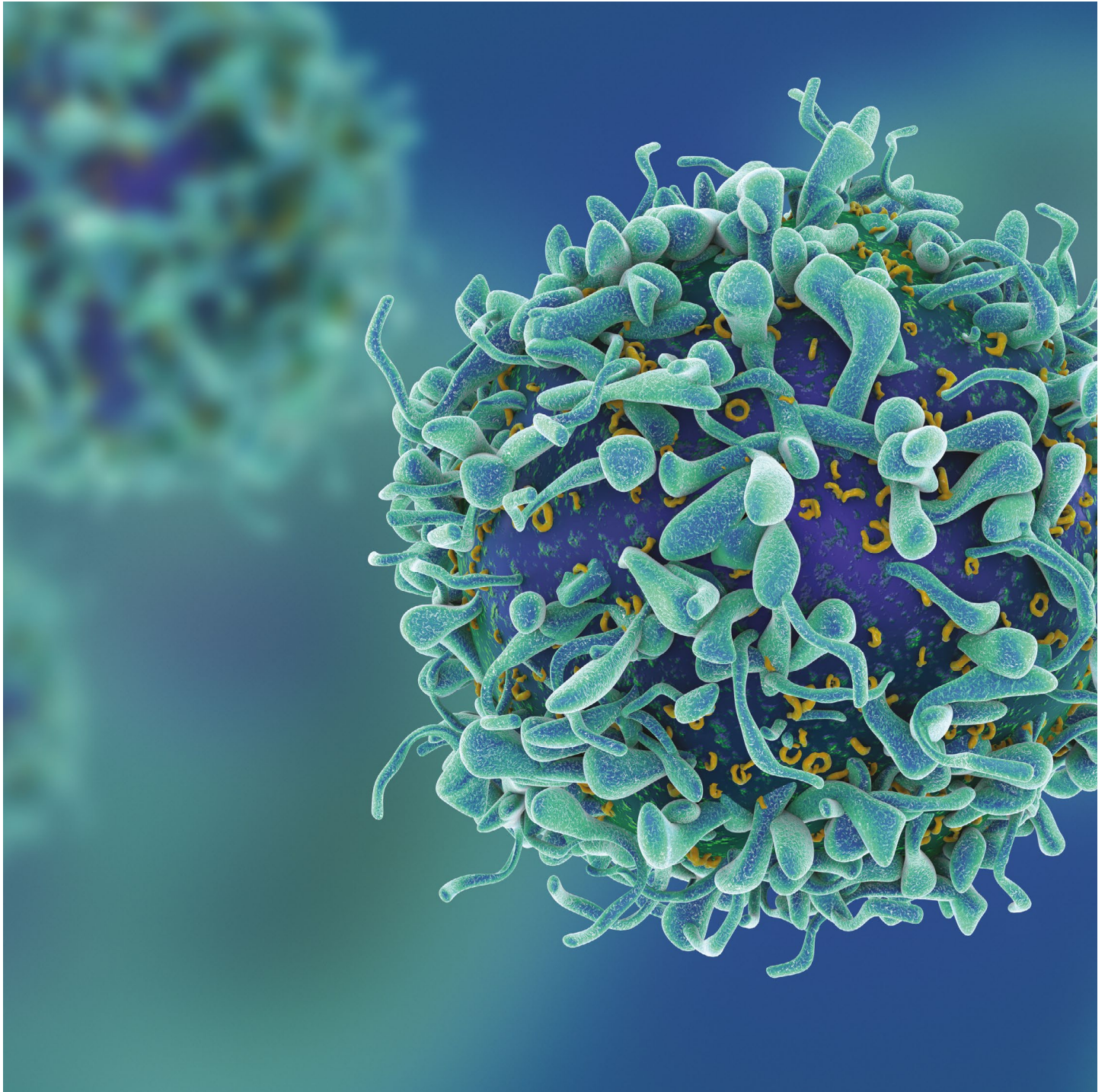


Cancer Cell Culture

PromoCell®

Solutions for
Cancer Research



Cancer Cell Culture

Our cancer cell culture portfolio covers a complete range of applications for culturing human malignant cells. Our innovative cancer cell culture solutions allow for culturing primary human cancer cells and cancer cell lines as 3D spheroids or 2D adherent monolayers. The xeno-free and serum-free formulation of our media allows for highly standardized cell culture conditions, and therefore ensures a high degree of reproducibility for your experiments.

Primary Cancer Culture System D-ACF

PromoCell's Primary Cancer Culture System D-ACF is a standardized, defined and animal component-free (D-ACF) system for the isolation and culture of human primary tumor cells. It supports the long-term culture of cancer cells while maintaining the clonal diversity of malignant subpopulations. Due to precise stromal control, prolonged culture allows for functional selection of malignant cells. This principle enables access to an enriched population of primary cancer cells, also suitable for the generation of cancer cell lines.

3D Tumorsphere Medium XF

The 3D Tumorsphere Medium XF has been designed to meet your requirements for the extended serial 3D tumorsphere culture. It supports the most commonly used cancer cell lines in tumorsphere/mammosphere culture. Continuous proliferation is supported during serial passage of 3D tumorsphere cultures. Thus, this culture system is also applicable for in vitro models of metastasis.

Cancer Cell Line Medium XF

The PromoCell Cancer Cell Line Medium XF was designed with the aim of achieving a universal, consistent and xeno-free environment for culturing most commonly used human cancer cell lines in 2D. It has no ill-defined components such as fetal calf serum, extracts or hydrolysates and exhibits very low lot-to-lot variability. Being broadly usable across all common adherently growing cancer cell lines, this culture medium is a cost-effective solution for ensuring efficient, genuinely standardized routine cultures.

Tab. 1: Overview of the PromoCell Cancer Cell Media Portfolio and their applications.

	Primary Cancer Culture System D-ACF	3D Tumorsphere Medium XF	Cancer Cell Line Medium XF
Product Specification			
Main application	Primary cancer cell isolation maintenance of heterogeneity	Extended 3D tumorsphere culture of cancer cell lines/enrichment of CSCs	Adherent long-term expansion of cancer cell lines
Culture pattern	2D/3D	3D	2D
Formulation	Defined formulation, animal-component free	Serum-free, xeno-free	Serum-free, xeno-free
Additional supplementation required	No	No	No
Plasticware	TC-treated	Suspension Culture	TC-treated
Culture surface treatment required	NCCD-Reagent (incl.)	No	Human fibronectin (C-43060; not included)
Product Application			
Selective isolation of malignant cells from primary tumors	✓	✗	✗
Selective isolation of malignant cells from PDX	✓	✗	✗
Limited stroma support	✓	✗	✗
Prevention of stroma overgrowth	✓	✗	✗
Enrichment of malignant cells from established cultures	✓	✗	✗
Depletion of non-cancerous cells from established cultures	✓	✗	✗
Serial 3D tumorsphere culture of established cancer cell lines	✗	✓	✗
Serial 3D mammosphere culture of established cancer cell lines	✗	✓	✗
Serial 3D neurosphere culture of established cancer cell lines	✗	✓	✗
Mammosphere culture (primary mammary stem cells)	✗	✓	✗
Neurosphere culture (primary neural stem cells)	✗	✓	✗
Adherent culture of established cancer cell lines	✗	✗	✓
Establish cell lines from primary cancer cells isolated with PCCS	✗	✓	✓

2D/3D = 2-/3-dimensional, PCCS = Primary Cancer Culture System, TC = tissue culture, PDX = patient-derived xenograft, XF = xeno-free, CSCs = Cancer Stem Cells

Primary Cancer Culture System D-ACF

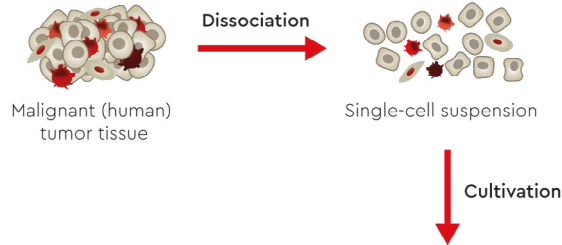
Key Advantages:

- Perform up to 5 isolations with one bottle of medium
- Maintains the diversity of original malignant tumor cell subpopulations
- Successfully deplete non-malignant cells from the culture
- No stromal overgrowth
- Defined and animal component-free (D-ACF)

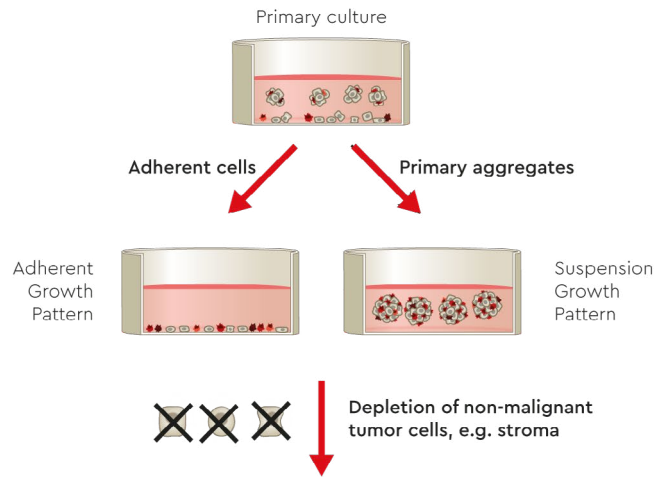
Recommended for:

- Tumor tissue samples
- Malignant/cancerous cells
- Established primary tumor cell cultures
- Patient-derived xenografts (PDX)

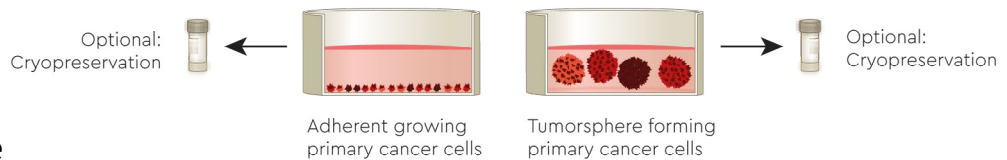
Day 0
Isolate



Weeks 2-4
Establish Primary Culture



Weeks 4-6
Enriched Malignant Cell Culture



Weeks 6 subseq.
Enhance Proliferation

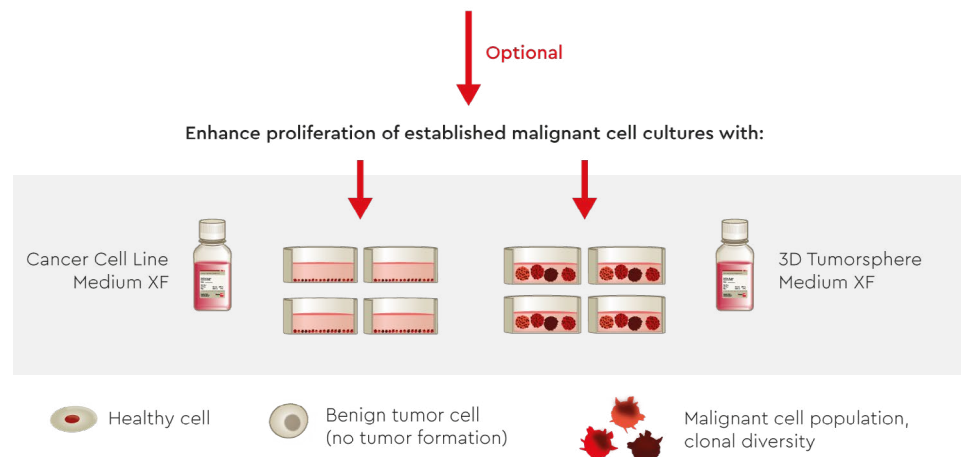


Fig. 1: Overview of the selective isolation of primary cancer cells from (human) tumor samples using the Primary Cancer Culture System. The PromoCell system makes it possible to reliably deplete non-malignant cells from the cell culture while supporting the maintenance of cancer cells and malignant cell heterogeneity. The optional establishment of cancer cell lines is indicated.

Primary Cancer Culture System D-ACF

Product Description:

The PromoCell Primary Cancer 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 selection process is achieved in a defined, animal-free culture environment without the use of cytotoxic agents. Since malignancy itself is employed as the exclusive functional selection cue, the culture system is applicable

to all types/entities of malignant cells, irrespective of their origin and the stage of the tumor. Provisional enrichment techniques, e.g. cell sorting and reliance on unassured markers, are thus rendered obsolete.

The PromoCell Primary Cancer Culture System is a complete culture system consisting of a bottle of Basal Medium, one vial of SupplementMix and one vial of NCCD-Reagent. One bottle of medium is typically sufficient for 3–5 primary isolations.

Primary cancer cells isolated with the Primary Cancer Culture System may also

be used for the generation of cancer cell lines by enhancing proliferation with the 3D Tumorsphere Medium XF (for cultures featuring a suspension growth pattern) or the Cancer Cell Line Medium XF (adherent growth pattern) (Fig. 1).

The Primary Cancer Culture System can also be used for other applications, e.g. enriching malignant subpopulation(s) in established cell lines or depleting of stromal cells and other non-cancerous cells from established primary cancer cell cultures.

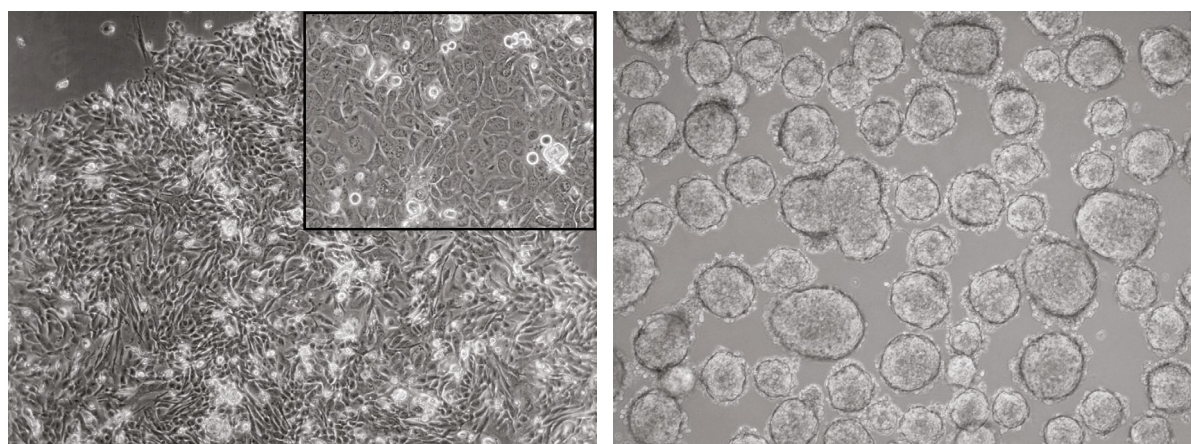


Fig. 2: Primary cultures after 4 weeks derived from human tumor samples with the Primary Cancer Culture System. Left: Primary culture derived from a squamous cell carcinoma featuring an adherent growth pattern. The upper right inset reveals cellular heterogeneity at a greater magnification. **Right:** Primary culture derived from a low-grade small cell lung cancer featuring a suspension growth pattern (3D sphere formation).

Primary Cancer Culture System

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 items

More information and detailed protocols available:

- Application Notes – Selective In Vitro Culture of Primary Human Cancer Cells from Human Tumor Samples; Isolation of Tumor Associated Macrophages (TAM) from Fresh Tumor Tissue (www.promocell.com/scientific-resources/application-notes)
- Poster – Primary Cancer Cell Culture from Human Tumor Sample (www.promocell.com/scientific-resources/posters)
- Website: www.promocell.com/product-category/cancer-cell-culture

3D Tumorsphere Medium XF

Key Advantages:

- Establish 3D tumorsphere cultures from 2D cancer cell lines
- Suitable for routine culture
- Works with most commonly used cancer cell lines
- Xeno-free and serum-free formulation

Recommended for:

- (Human) cancer cell lines (Tab. 2)
- Established tumorsphere cultures

Product Description:

The 3D Tumorsphere Medium XF supports the most commonly used cancer cell lines in tumorsphere/mammosphere cultures (Tab. 2). In contrast to the current adherent 2D culture of cancer cells, this type of 3D culture selectively exploits inherent biologic features of cancer stem cells (CSCs), such as anoikis resistance and self-renewal. It also provides a more physiological 3D microenvironment. Continuous proliferation is also supported during serial passage of 3D tumorsphere

cultures (see Fig. 3B, C). Thus, this culture system is also applicable for *in vitro* models of metastasis. In addition, the xeno-free and serum-free formulation provides a standardized culture devoid of stimuli of uncharacterized origin. This is a significant benefit in terms of CSCs which are a population of highly responsive stem cells requiring reliable and reproducible control of the self-renewal/differentiation axis.

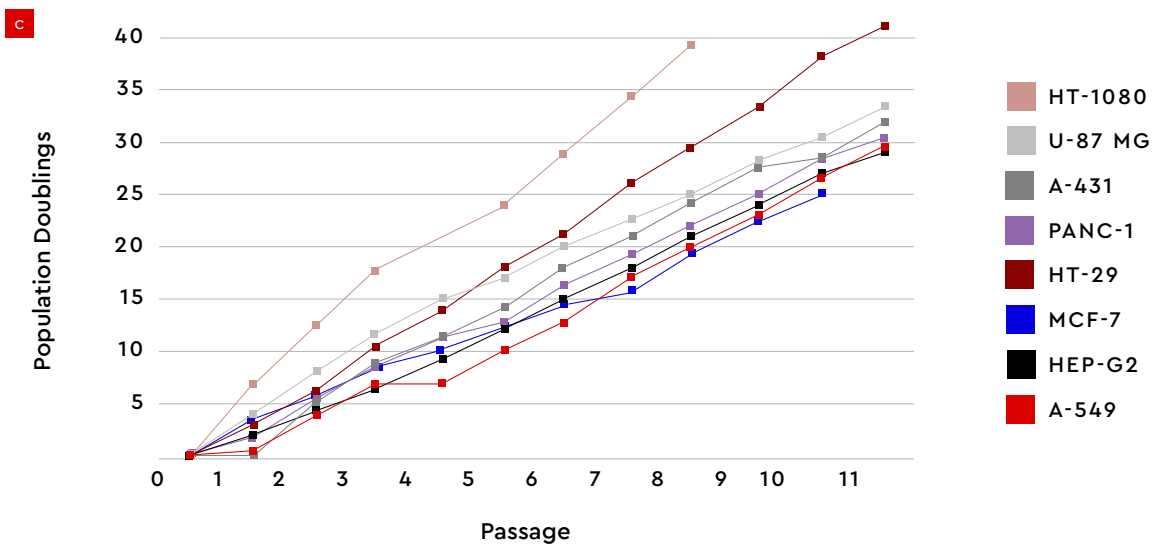
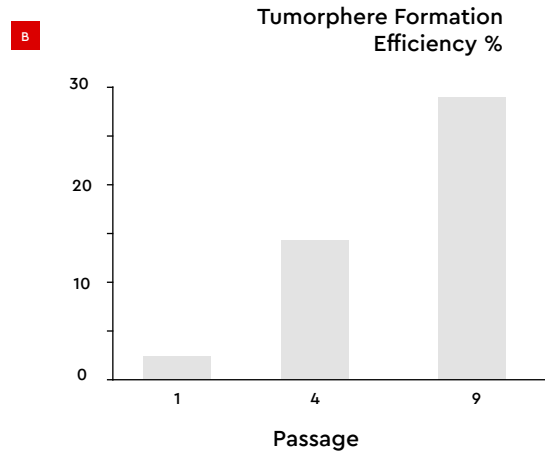
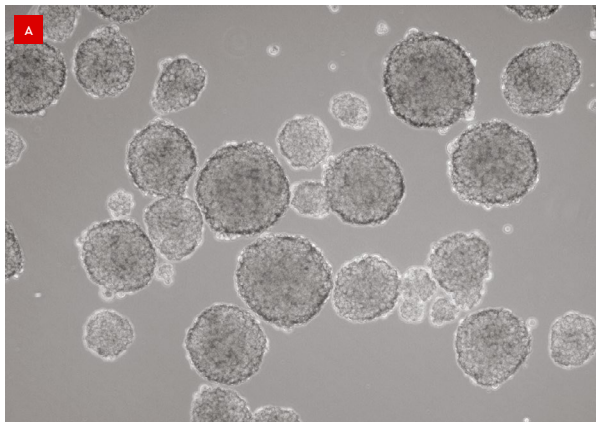


Fig. 3: 3D tumorsphere cell culture of cancer cells and quantifications. **A:** HT1080 fibrosarcoma cells after 10 serial passages. **B:** Serial passage of MCF-7 cells in the 3D Tumorsphere Medium XF results in a significant increase of TFE from 2% in P1 to 28% in P9. **C:** Proliferation curves of various established cancer cell lines expanded by serial passage of 3D tumorsphere cultures.

3D Tumorsphere Medium XF

Tab. 2:
List of some cell types successfully tested for serial passage with the PromoCell 3D Tumorsphere Medium XF.

Tissue	Tested Cell Line	Cell Line Origin
Brain	U-87 MG	Grade IV glioblastoma / astrocytoma of the human brain
Breast	MCF-7	Pleural effusion of metastatic human breast adenocarcinoma
Breast	MDA-MB-231	Pleural effusion of metastatic human breast adenocarcinoma (triple-negative)
Colon	HT-29	Human colon adenocarcinoma
Connective Tissue	HT1080	Human fibrosarcoma
Liver	HepG@	Hepatocellular carcinoma of the human liver
Lung	A-549	Human lung carcinoma
Pancreas	Panc-1	Epithelioid carcinoma of the human pancreatic duct
Prostate	LNCap	Lymph node metastasis of human prostate adenocarcinoma
Skin	A-431	Epidermoid carcinoma of the human skin



3D Tumorsphere Medium XF

Product	Size	Catalog Number
3D Tumorsphere Medium XF	250 ml	C-28070
3D Tumorsphere Medium XF, phenol red-free	250 ml	C-28075

More information and detailed protocols available:

- Application Notes – Tumorsphere Culture of Cancer Stem Cells (CSC) with the PromoCell 3D Tumorsphere Medium XF; Determination of the Tumorsphere Formation Efficiency (TFE) with the PromoCell 3D Tumorsphere Medium XF; Extended Mammosphere Culture of Human Breast Cancer Cells; Extended Neurosphere Culture of Brain Tumor Stem Cells with the PromoCell 3D Tumorsphere Medium XF (www.promocell.com/scientific-resources/application-notes)
- Posters – 3D Tumorsphere Culture Model; Tumorsphere Cell Culture – Method and Its Efficiency (www.promocell.com/scientific-resources/posters)
- Website (www.promocell.com/product-category/cancer-cell-culture)

Cancer Cell Line Medium XF

Key Advantages:

- Robust growth performance for standard 2D cell culture
- Compatible with most commonly used cancer cell lines
- Xeno-free and serum-free formulation

Recommended for:

- (Human) cancer cell lines

Product Description:

The Cancer Cell Line Medium XF has no undefined components such as fetal calf serum (FCS), extracts or hydrolysates and exhibits very low lot-to-lot variability. Traditionally, established cell lines have been propagated almost exclusively in standard culture media supplemented with significant amounts of FCS. However, poorly defined culture media components such as FCS are a well-known and significant source of variations and unwanted physiological, genetic and epigenetic effects. These variations may endanger the reliability of

experimental results. The Cancer Cell Line Medium XF provides a xeno-free and serum-free culture environment which is key for obtaining more accurate results with cell line models and facilitates subsequent data analysis and interpretation. 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 and exploiting the unique features of permanent cell lines as *in vitro* research models.

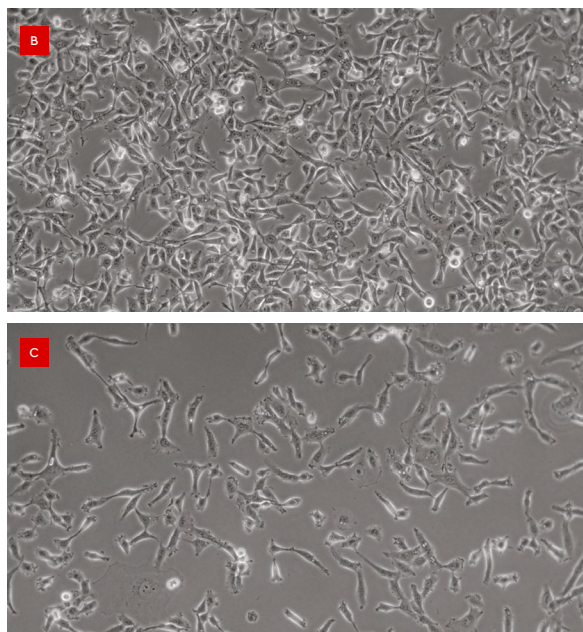
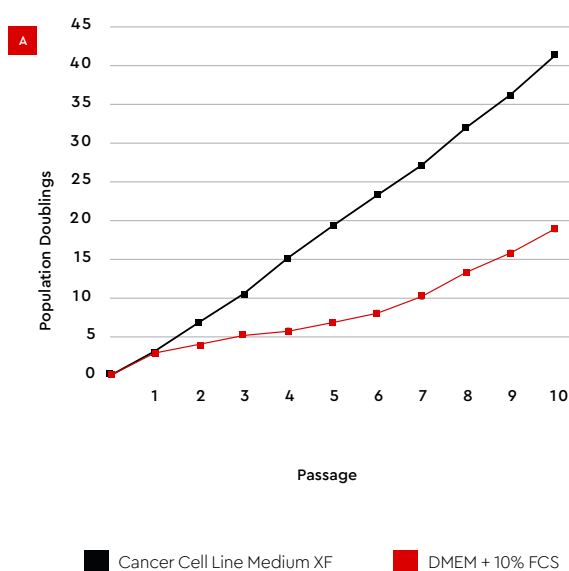


Fig. 4

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).

Cancer Cell Line Medium XF

Product	Size	Catalog Number
Cancer Cell Line Medium XF	250 ml	C-28077
Fibronectin solution, human (1 mg/ml)	5 ml	C-43060



More information and detailed protocols available:

- Application Note – Standardized Culturing of Established Cancer Cell Lines in Xeno-Free Conditions with PromoCell's Cancer Cell Line Medium XF (www.promocell.com/scientific-resources/application-notes)
- Website (www.promocell.com/product-category/cancer-cell-culture)

Related Products:

Media, Buffers and Reagents	Size	Catalog Number
Accutase-Solution	100 ml	C-41310
Cryo-SFM	30 ml / 125 ml	C-29910 / C-29912
DetachKit	3 × 125 ml	C-41210
Dulbecco's PBS, w/o Ca ⁺⁺ /Mg ⁺⁺	500 ml	C-40232
Fibronectin solution, bovine (1 mg/ml)	5 ml	C-43050
Fibronectin solution, human (1 mg/ml)	5 ml	C-43060

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