

# Tumorsphere Culture of Cancer Stem Cells (CSC) with the PromoCell 3D Tumorsphere Medium XF



## Application Note

### The PromoCell 3D Tumorsphere Medium XF

The PromoCell 3D Tumorsphere Medium XF has been designed to meet your requirements for the extended serial 3D tumorsphere culture.

This 3D Tumorsphere Medium XF supports the most commonly used cancer stem cell lines in tumorspheres/mammospheres culture.

In contrast to the current adherent 2D

culture of cancer cells, this type of 3D culture selectively exploits inherent biologic features of CSC, such as anoikis resistance and self-renewal. Continuous proliferation is also supported during serial passage of 3D tumorsphere cultures (see Fig. 1). Thus, this culture system is also applicable for *in vitro* models of metastasis.

PromoCell's 3D Tumorsphere Medium XF is ready-to-use and xeno-free, providing a standardized culture devoid

of stimuli of uncharacterized origin. This is a significant benefit in terms of CSC which are a population of highly responsive stem cells requiring reliable and reproducible control of the self-renewal/differentiation axis.

The xeno-free PromoCell 3D Tumorsphere Medium XF is suitable for the cost-efficient and standardized routine culture of tumorspheres/mammospheres for a wide range of cell lines (Tab. 1).

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	HepG2	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

Tab. 1: List of cell types tested for serial passage with the PromoCell 3D Tumorsphere Medium XF.

### Background

"The term 'malignancy' refers to cancerous cells that have the ability to spread to other sites in the body (metastasize) or to invade nearby (locally) and destroy tissues. Malignant cells tend to have fast, uncontrolled growth and do not die normally due to changes in their genetic makeup.

Malignant cells that are resistant to treatment may return after all detectable traces of them have been removed or destroyed." (Medline Encyclopedia)

### History of cancer

Paleopathologic findings indicate that cancer has been recognized for millen-

nia. The first written documentation of human cancer dates from 3000 BC, with the first description of metastasis going back to the year 50 AD [1]. More than 5000 years later, many types of cancer still remain incurable and prove fatal, particularly in cases of recurrence after seemingly successful primary treatment. In 1863, almost 160 years ago, the pathologist Rudolf Virchow was the first to propose the model of cancer stem cells stating that 'immature cells' represent the origin of cancer [2]. In 1959 Pierce introduced the term 'cancer stem cell' [3], while in 1997 Bonnet described a specific subpopulation of CSC as 'cancer driver cells' in myeloid leukemia [4]. Recently, CSC have been identified in various cancers including hematopoietic malignancies and a range of solid tumors [5].

### Research and therapy for malignancy

Cancer research is one of today's major research fields producing thousands of publications every year. The most frequently studied malignancies are cancers of the breast, lung, liver, colon, skin, pancreas and nervous system. Traditional therapeutic approaches aim to eliminate as much of the tumor mass as possible by means of surgery, irradiation, chemotherapy and biologics. However, accumulating evidence suggests these measures target the more harmless, rapidly dividing cell mass of the tumor and do not eradicate the putative root of the disease - CSC.

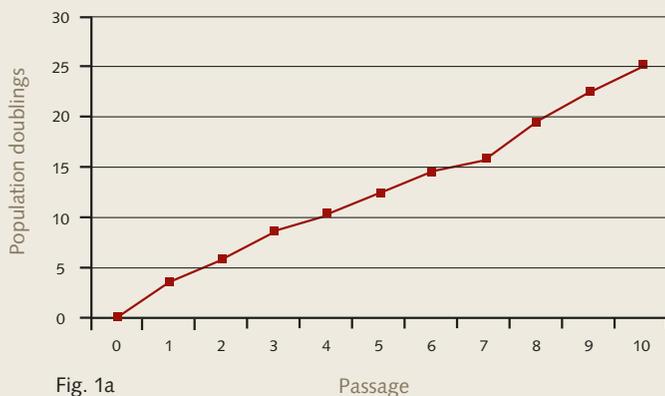


Fig. 1a

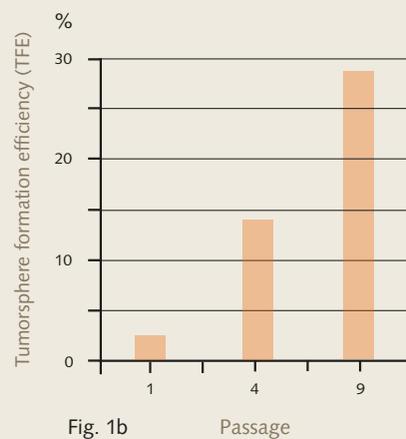


Fig. 1b

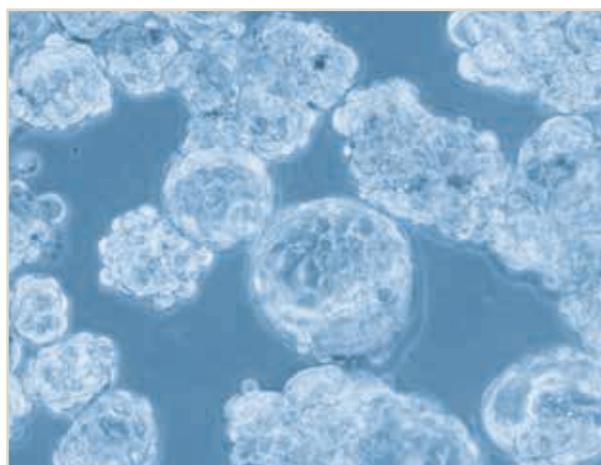
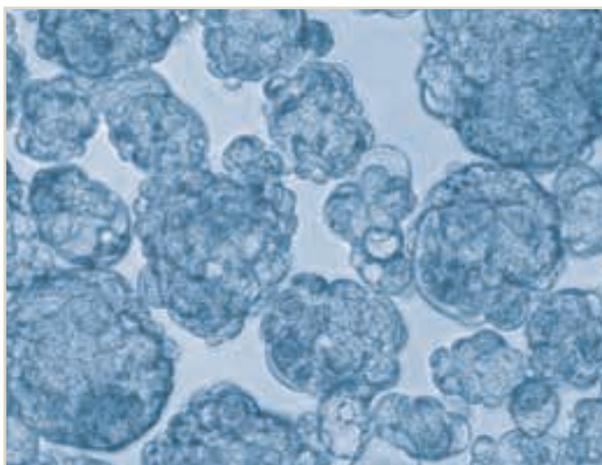
**Fig. 1a: Plot of cumulative population doublings of MCF-7 cells during serial passage of 3D tumorsphere culture.** Forty thousand MCF-7 cells per well (10,000/ml) were plated in triplicate in the PromoCell 3D Tumorsphere Medium XF using 6-well suspension culture plates. Serial passage by enzymatic dissociation according to the protocol was performed every 9 days. Tumorsphere formation and proliferation were maintained during the culture, which was discontinued after passage 10 with no sign of growth rate inhibition. The MCF-7 tumorsphere culture achieved approximately 2.5 population doublings per passage. The proliferation rate is dependent on cell line and may vary accordingly with other types of tumor cells.

**Fig. 1b:** Serial passage of MCF-7 cells in the 3D Tumorsphere Medium XF results in significant increase of TFE from 2% in P1 to 28% in P9, respectively.

In the cancer stem cell model of tumors, CSC are defined as a small subset of malignant cells with the exclusive ability to self-renew and maintain the tumor. They can differentiate into a heterogeneous mass of non-tumorigenic cancer

cell types, which usually constitute the majority of the tumor [6]. It is clear in this context that CSC, despite their malignant phenotype, share common hallmarks of normal stem cells (Fig. 3), assigning extraordinary biologic potential

to these cells. These combined capabilities account for one of the greatest risks in the treatment of malignancies: metastasis.



**Fig. 2: Tumorsphere culture of MCF-7 mammary carcinoma cells in the PromoCell 3D Tumorsphere Medium XF (C-28070) after 10 serial passages.** The tumorsphere culture was subjected to serial passage every 9 days by enzymatic dissociation according to the protocol. Robust tumorsphere formation was maintained during serial culture. See Fig. 1 for proliferation data.

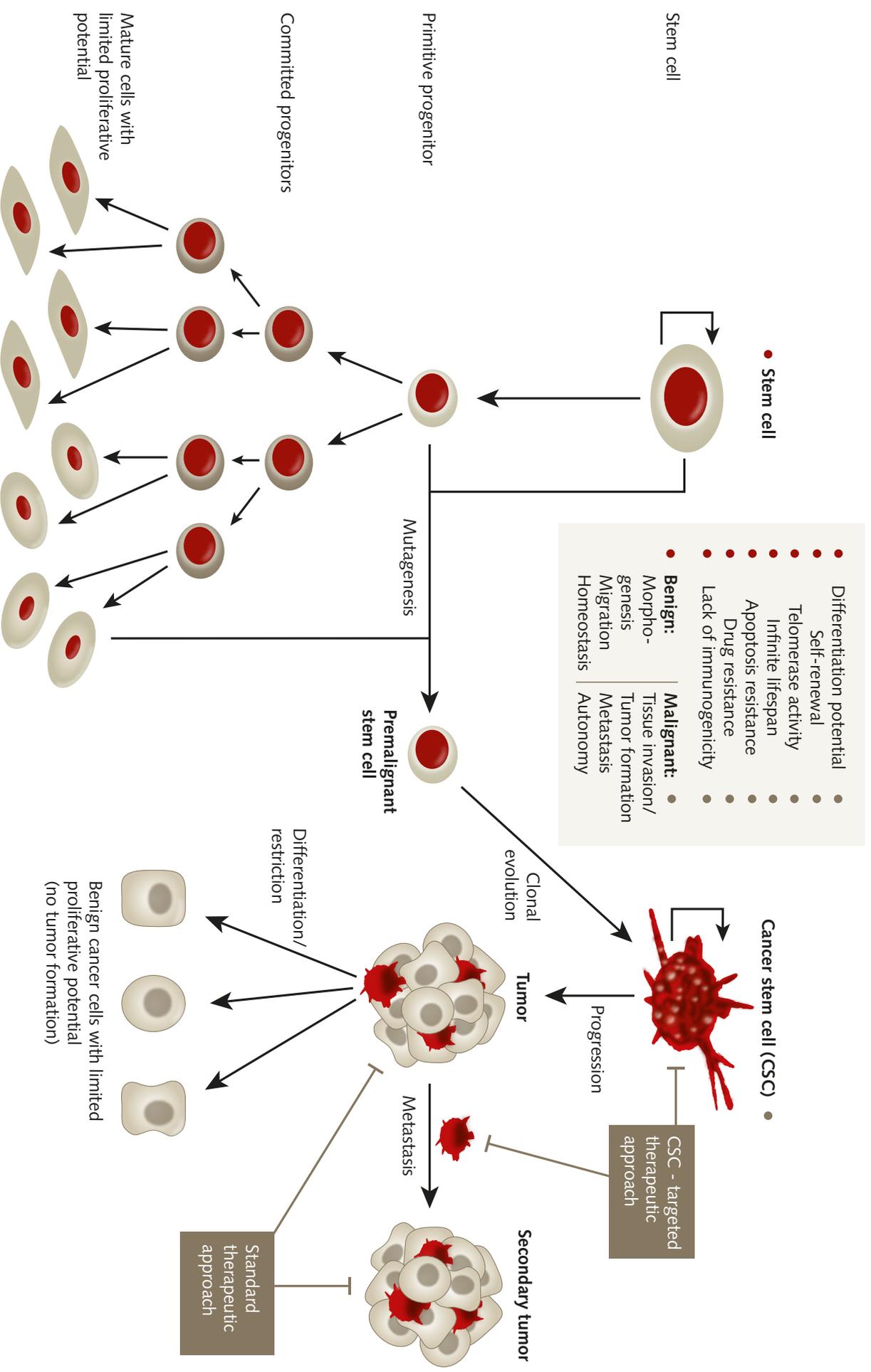


Fig. 3: Schematic overview of the origin, evolution and fate of cancer stem cells (CSC).

CSC are self-sustaining and largely resistant to anoikis, the term for apoptosis induced by the detachment of adherently growing cells from the extracellular matrix. Therefore, CSC can separate from the primary tumor, travel and spread through the body where they may form secondary tumors (metastases) in distant organs. Metastases may develop quickly or over several years after seemingly successful treatment of the primary tumor. It is believed that relapse is caused by quiescent CSC that are able to evade current therapeutic regimens by using protective mechanisms mediated by their stem cell properties.

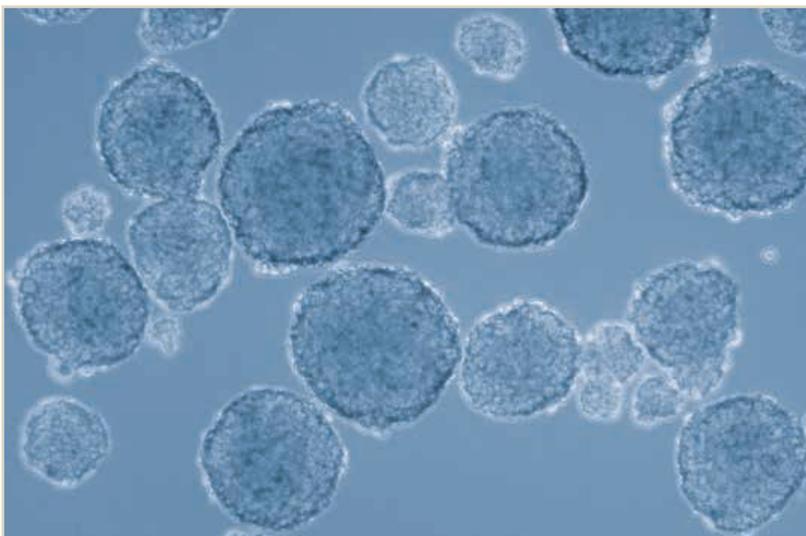
It is clear that cancer research needs to be realigned, especially when exploring new clinical strategies to treat malignancies. CSC are now considered to be a new therapeutic target and it is believed that their elimination could lead to permanent remission or even cure. This might be achieved by direct eradication of the CSC or by specific adaptation of CSC cell division from asymmetric to symmetric leading to elimination of the CSC population by blocking their self-renewal capabilities [6, 7]. For this to be achieved, detailed characterization is required.

### Characterization of cancer stem cells

Various approaches have been undertaken to characterize CSC based on marker detection. These include staining of intra- and extracellular molecules, as well as measuring the activity of certain cellular enzymes, such as aldehyde dehydrogenase (ALDH1) or small molecule transporters like the ABC transport system [8–10]. However, research is hindered by the heterogeneity of CSC in different cancers [6, 11], as well as a lack of specificity, consistency and correlation of established markers with functional CSC features, such as tumorigenesis [6, 12]. Thus, a robust, reliable, and notably holistic, marker-based method for CSC detection and characterization has seemed a distant prospect. As a result, currently the greatest obstacle in CSC research is the isolation and purification of sufficient numbers of functional, homogenous CSC populations. At present, CSC can only be defined experimentally by their ability to recapitulate generation of a continuously growing tumor [6].

To date, the most accepted strategies for the analysis of CSC are generally

based on the detection of their basic functional features. These include typical stem cell properties, such as self-renewal and pluripotency, as well as specific hallmarks of cancer, such as serially transferable tumorigenic potential and anoikis resistance. However, lack of a primary model system means that research is forced to rely on indirect readouts generated by alternative model systems and tests. The gold standard functional *in vivo* assay for CSC is the serial transplantation into orthotopic sites of immunocompromised mice. However, this is laborious and results can be difficult to interpret. Established *in vitro* methods include assays for serial colony-forming units (CFU) in semi-solid media and label-retention as well as testing for the formation of serially passageable tumorspheres in a 3D suspension culture (see Figs. 2 and 4) [6]. Derived from neurosphere culture techniques of neural stem cells, the advantage of serial tumorsphere culture is the option for simultaneous determination of the sphere formation efficiency. This provides quantitative information on the temporal CSC ratio during culture on an exclusively functional basis.



**Fig. 4: Tumorsphere culture of HT1080 fibrosarcoma cells in the PromoCell 3D Tumorsphere Medium XF (C-28070) after 10 serial passages.** The tumorsphere culture was subjected to serial passage every 6 days by enzymatic dissociation according to the protocol. Robust tumorsphere formation was maintained during serial culture.

*Use aseptic techniques and a laminar flow bench.*

## Tumorsphere culture protocol

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### I. Materials

- 3D Tumorsphere Medium XF (C-28070 or C-28075)
- Phosphate Buffered Saline w/o Ca<sup>++</sup>/Mg<sup>++</sup> (PBS, C-40232)
- Detach-Kit (C-41210)
- 6-well Suspension Culture Plates (e.g. Greiner Bio One, No. 657 185)
- Adherently growing human cancer cells (for initial tumorsphere culture set-up)

### A) Initiation of the tumorsphere culture

#### 1. Harvest the adherent cells

Detach the cells of a human CSC-containing adherently growing cancer cell line using your standard procedures. The cells should be 80–90% confluent and in good condition. Centrifuge the cell suspension for 5 minutes at 300 x g and aspirate the supernatant. Resuspend the cells in a small volume, e.g. 3–5 ml, of the PromoCell 3D Tumorsphere Medium XF.

#### 2. Count the cells

Count the cells using your routine method and adjust the volume with PromoCell 3D Tumorsphere Medium XF to obtain a concentration of 1 million cells/ml.

#### 3. Set up the tumorsphere culture

Seed the cells in appropriate suspension culture vessels at 10,000 cells/ml, e.g. 40,000 cells in 4 ml of PromoCell 3D Tumorsphere Medium XF in each well of a 6-well suspension culture plate.

#### 4. Allow the tumorspheres to grow

Incubate the culture for 4–10 days, depending on the cell type used. Add one-half of the culture volume of fresh PromoCell 3D Tumorsphere Medium XF every 3–4 days. Do not change the medium.

#### 5. Passage of the tumorsphere culture

The tumorspheres should be passaged (section B below) before they start to develop a dark center. Depending on the cell type used optimal passage should occur after 4–10 days.

### B) Serial passage of tumorsphere cultures

#### 1. Collect the tumorspheres

Transfer the PromoCell 3D Tumorsphere Medium XF containing the tumorspheres into 15 ml conical tubes using a serological pipet.

#### 2. Gravity sedimentation of the tumorspheres

Allow the spheres to settle by gravity sedimentation for 10 minutes at room temperature. Aspirate the supernatant, but leave approximately 200 µl in the conical tube. Do not aspirate the tumorspheres.

#### 3. Wash the tumorspheres

Repeat the sedimentation (step 2 previous page) with an equal volume of PBS. Gently aspirate the PBS leaving approximately 200 µl in the conical tube.

## *Tumorsphere Culture Protocol*

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#### 4. Enzymatic digestion of the tumorspheres

Add 1 ml of Trypsin-EDTA to the tumorspheres and incubate for 2–4 minutes at room temperature. Keep the spheres resuspended in the trypsin solution by pipetting up and down once every 30 seconds. Avoid sedimentation of the spheres.

**Note:** The optimal incubation time required to achieve complete dissociation in step B5 (below) must be determined empirically by the user for each cell type. While 2–3 minutes will be optimal in most cases, tumorspheres of some cell types, e.g. MCF-7, may need longer incubation, especially in higher passages. If a completely defined dissociation process is preferred, a recombinant trypsin solution may be used as an alternative dissociation reagent according to the supplier's instructions.

#### 5. Break down remaining cell aggregates

Pipet the spheres up and down 10–20 times using a 1000 µl pipet tip to generate a single cell suspension. Aspirate the cell suspension as normal but tilt the pipet tip slightly at the bottom of the tube when expelling the cells. The shear forces generated facilitate the break up of any residual cell aggregates. Perform a visual check to confirm that no large cell aggregates remain.

Immediately after trituration, add twice the volume of Trypsin Neutralization Solution (TNS).

**Note:** Do not over-triturate as cell viability will be compromised. If in doubt, monitor the dissociation process microscopically. Non-dissociated cell aggregates may be removed by passing the cell suspension through a 40 µm cell strainer. When using recombinant trypsin use fresh PromoCell 3D Tumorsphere Medium XF for inactivation instead of TNS.

#### 6. Determine the cell number and viability

Make up to 5 ml with fresh PromoCell 3D Tumorsphere Medium XF and determine the cell number and viability. Centrifuge the cells for 5 minutes at 300 x g. Discard the supernatant and resuspend the cells in fresh 3D Tumorsphere Medium XF at 1 million cells/ml.

**Note:** Alternatively, the cells may be resuspended in buffer, e.g. PBS w/o Ca<sup>++</sup>/Mg<sup>++</sup> plus 0.5% albumin plus 2 mM EDTA, and used for further experiments and/or analytical procedures.

#### 7. Plate the Cells

Reseed the cells at 10,000 cells/ml in new suspension culture vessels. Typically, 6-well plates with 40,000 cells in 4 ml of medium per well are used.

## *Tumorsphere Culture Protocol*



## Products

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

## Related Products

Product	Size	Catalog Number
Dulbecco's PBS, w/o Ca <sup>++</sup> /Mg <sup>++</sup>	500 ml	C-40232
DetachKit	3 x 125 ml	C-41210

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