3D Tumorsphere Medium XF

PromoCell

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

Recommended for

- Sphere-forming cancer cell lines
- Cancer stem cells (CSCs)

Product Description

The PromoCell 3D Tumorsphere Medium XF is a culture system tested for the standardized serial culture of cancer cell lines as tumorspheres / mammospheres. The formulation supports sustained cell proliferation allowing for serial passage of the 3D culture.
The PromoCell 3D Tumorsphere Medium XF is ready-to-use and xeno-free providing a culture environment devoid of all stimuli originating from non-defined materials. It is particularly suitable as a cost-efficient tool for the standardized routine-culture of tumorspheres / mammospheres.

PromoCell 3D Tumorsphere Medium XF consists of a bottle of Basal Medium and one vial of SupplementMix. Adding the SupplementMix to the Basal Medium results in the complete medium.

Supplementation Details

PromoCell 3D Tumorsphere 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 4 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 4 to 8°C. For use, pre-warm only an aliquot of the complete medium and keep the remaining medium refrigerated at 4 to 8°C.

Quality Control

All lots of the PromoCell 3D Tumorsphere Medium XF are subjected to comprehensive quality control tests using human mammary cancer cells. Each lot of PromoCell 3D Tumorsphere Medium XF is tested for the ability to support tumorsphere/mammosphere formation, proliferation and serial passage of the 3D culture.
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.

Tumorsphere Culture Protocol

Tumorsphere Culture Protocol

I. Materials

- 3D Tumorsphere Medium XF (C-28070 or C-28075)
- Phosphate Buffered Saline w/o Ca^{++}/Mg^{++} (PBS, C-40232)
- DetachKit (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 medium 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.

Use aseptic techniques and a laminar flow bench.

Tumorsphere Culture Protocol

3. **Wash the tumorspheres**
   Repeat the sedimentation (step 2 above) with an equal volume of PBS. Gently aspirate the PBS leaving approximately 200 µl in the conical tube.

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 medium at 1 million cells/ml.
   Note: Alternatively, the cells may be resuspended in buffer, e.g. PBS w/o Ca^{++}/Mg^{++} 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.

PromoCell GmbH

Sickingenstr. 63/65
69126 Heidelberg
Germany

Email: info@promocell.com
www.promocell.com

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 – 90 93 32 (ligne verte)
Téléfax: 0800 – 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

09/2018