3D Tumorsphere Medium XF

PromoCell®

Instruction manual

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)
- Tumor biopsies

Product description

As a part of our Cancer Media Toolbox, our 3D Tumorsphere Medium XF is ideal for isolation and 3D long-term cultivation of CSCs from tumor biopsies and cancer cell lines. The formulation supports enrichment and maintenance of cancer stem cells as well as further differentiated cancer cells with high cell proliferation rates, allowing for serial passage of the 3D culture.

The 3D Tumorsphere Medium XF is a synthetic growth medium. It's ready-to-use, serum-free, and xeno-free formulation provides 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.

Our 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

The 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 the supplements are added to the basal medium, store at 4–8°C and use within 6 weeks. 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 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 and bacteria).

Intended use

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

Tumorsphere culture protocol

I. Initiation of the tumorsphere culture

Use aseptic techniques and a laminar flow bench.

Materials

- 3D Tumorsphere Medium XF (C-28070 or C-28075)
- Phosphate Buffered Saline without Ca^{++}/Mg^{++} (PBS, C-40232)
- DetachKit (C-41210)
- 6-well suspension culture plates (e.g., Greiner Bio-One, N°. 657 185)
- Adherently growing human cancer cells (for initial tumorsphere culture set-up)

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 3D Tumorsphere Medium XF.

2

Count the cells

Count the cells using your routine method and adjust the volume with 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 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 to 10 days, depending on the cell type used. Add one half of the culture volume of fresh 3D Tumorsphere Medium XF every 3 to 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 to 10 days.

II. Serial passage of tumorsphere cultures

Use aseptic techniques and a laminar flow bench.

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.

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 to 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 5 (below) must be determined empirically by the user for each cell type. While 2 to 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 breakup 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 3D Tumorsphere Medium XF for inactivation instead of TNS.

6

Determine the cell number and viability

Make up to 5 ml with fresh 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 without 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

If you require special media modifications, we offer a custom media service starting at 10 bottles per order.
Contact us at info@promocell.com to find out more.

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