Technical Information

Tumorsphere culture of human cancer cell lines using Cancer Stem Premium™ medium

Introduction

Cancer stem cells (CSC's) reside within the normal tumor cell milieu, and retain certain characteristics of normal stem cells. As such CSC's can be viewed as precursor-type cells capable of driving tumor growth, have been linked with increased malignancy and metastatic potential, being highly tumorigenic in contrast to other cancer cells, persist in tumors as a distinct population, and may cause relapse by giving rise to new tumor growth with increased metastatic potential.

CSC can self-renew, and differentiate into multiple cell types normally found in clinical tumor samples. Specific CSC cell surface proteins, in addition to normal stem cell surface markers, such as Sca-1, CD34, CD133, and CD117, also include CD44, CD24, CD20, CD105, and CD326 (EpCAM), which have been found to be either under-expressed, or over-expressed, depending on the tumor type.

Identification and isolation of CSC's from either tumor cell line spheroids in vitro, or in vivo tumors, will provide valuable cell populations in order to study their origin and mechanisms of establishment, maintenance, and importantly the molecular mechanisms responsible for their conversion. This will therefore provide an arsenal for the development of CSC-specific targeted therapies, to increase survival rates amongst cancer patients in general, but especially for those at risk from tumors with high metastatic potential.

Pluripotent, progenitor CSC's, and their ability to direct the growth and proliferation of new tumor masses, even following therapeutic intervention, has led Promab, with it's long history of mon°Clonal antibody development to aggressively pursue programs to develop CSC marker-specific antibodies. These antibodies may then be used as research or direct therapeutic tools. A direct consequence of this strategy has led to Promab's development of CSC-specific cell culture pr°Cedures, and products to allow for CSC isolation and propagation as tumor spheres.

Generating a Single Cell Suspension

Starting from a confluent (90%) culture in a T75 tissue culture flask.

Disruption of Adherent Cells

1. Remove existing medium, and rinse adherent cells with 5 mL of sterile Hanks Balanced Salt Solution (HBSS), or Phosphate Buffered Saline (PBS); discard wash (this will remove trace amounts of fetal bovine serum (FBS), which will normally may inhibit the action of trypsin)
2. Add 5 mL of pre-warmed (37°C) Trypsin:EDTA, and place the flask in a 37°C incubator for 2 min.
3. Following incubation cells should be loosely attached to the flask surface, and cells are disrupted by repeated pipetting (using a 5 mL sterile pipette)

The Trypsin: EDTA incubation time is critical to avoid excess clumping and cell death. While 2 min will work for many cell types/lines, this may need to be optimized based on the specific cell type/line being used.

4. Following disruption, immediately add 15 mL of DMEM media, containing 10% FBS, to the trypsinized cells, transfer to a sterile 50 mL polypropylene conical centrifuge tube, and pellet cells by centrifugation at 1000 RPM for 5 min.
5. Resuspend cells in 10 mL of Cancer Stem Premium media.
6. The cell suspension is passed through a sterile 20 µM cell strainer to remove any cell clumps remaining in the cell suspension.
7. Remove a 10 µL aliquot for cell counting.

Plating a Single Cell Suspension for Tumorsphere Formation

96 well plates

8a. From the single cell suspension population seed 4000 cells into each well of a 96 well tissue culture plate.
9a. Plates are then placed in a 37°C/5%CO2 incubator.
10a. Cultures are maintained by adding an additional 10% of the total well volume every two days, over a period of 7-10 days.
11a. Tumorspheres > 50 uM in size are then counted, and may be passaged for further experimentation

6 well plates

8b. From the single cell suspension population seed 50,000 cells into each well of a 6 well tissue culture plate.
9b. Plates are then placed in a 37°C/5%CO2 incubator.
10b. Cultures are maintained by adding an additional 10% of the total well volume every two days, over a period of 7-10 days.
11b. Tumorspheres > 50 uM in size are then counted, and may be passaged for further experimentation

Seeding Cell Number

Cell Line	96-well Plate	6-well Plate
MCF7	4,000 cells/well	50,000 cells/well
SKOV3	2,000 cells/well	40,000 cells/well
PLC/PRF/5	4,000 cells/well	40,000 cells/well
HCT116	2,000 cells/well	40,000 cells/well
HT1080	2,000 cells/well	40,000 cells/well

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Disruption and Passaging of Tumorspheres.

The following protocol is designed for collection and passaging of tumorspheres from a single 6-well plate. The same protocol has been expanded to generate and passage cells from tumorpsheres from four 6-well plates, with no change in viability, or subsequent generation of next-generation tumorspheres.

1. Tumorsphere cultures are incubated for 7-10 days in Cancer Stem Premium™ (catalog no. 20101-100, or 20141-500) media in each of six wells, for a single 6-well plate (tumorspheres, from a suspension of single cells, are visible at approximately 3 days). The majority of tumorspheres at this point will be approximately 50 mm in diameter.
2. The entire culture volume, from all wells (2 mL per well), are transferred to a sterile 50 mL polypropylene centrifuge tube and centrifuged for 10 minutes at 800 rpm.
3. Carefully remove all supernatant, as pellet at this stage may be loose.
4. To the pellet, which from the complete contents of one 6-well plate will be approximately 0.2 mL, is added 0.5 mL of pre-warmed (37°C) Trypsin:EDTA (for 4 x 6-well plates, pellet is approximately 0.5 – 0.75 mL, and 1 mL of pre-warmed (37°C) Trypsin:EDTA is added).
5. Using a sterile P1000 tip (2 mL pipette for 4 x 6-well plates), the tumorspheres are mechanically disrupted. Verification of disruption of spheres is performed microscopically.

Should disruption prove difficult, cultures may incubated with trypsin:EDTA for an additional 1 min.

6. Once disruption is complete, add 15 mL of DMEM/10% FBS media to the culture, and centrifuge for 10 minutes at 800 rpm.
7. Remove the media supernatant, and resuspend the pellet in 24 mL of Cancer Stem Premium™ media. Remove an aliquot of cells to perform a viability count, and as soon as it is confirmed that the culture contains a minimum of 90% viable cells, 2 mL are plated per well of a new 6-well plate.
8. Cells are then incubated at 37°C/5% CO2 for an additional 7-10 days.

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