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Isolation of cancer stem cells by sphere formation assay

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1 Works for me dx.doi.org/10.17504/protocols.io.bdt8i6rw

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ABSTRACT

Cancer stem cells (CSCs) are a small subpopulation of tumor cells that are thought to be responsible for recurrence and metastasis of cancer due to their ability for self-renewal and differentiation into multiple cancer cell types.

CSCs are also known to play a key role in the development of metastases. Cancer cells undergoing Epithelial to Mesenchymal Transition (EMT) have also been shown to attain CSC phenotypes and acquire invasive and migratory properties. Therefore, understanding the characteristics of CSCs may help to develop better treatment strategies.

The sphere formation assay has been widely used to isolate CSCs using anchorage-independent sphere culture. CSCs can grow on ultra-low attachment plates that are coated with a layer to inhibit the attachment of cells. When cells are grown in serum-free and non-adherent conditions, CSCs can survive and clonally expand to form spheres, whereas differentiated tumor cells undergo apoptosis due to their anchorage dependence.

MATERIALS

NAME	CATALOG #	VENDOR
Penicillin-Streptomycin	15140122	Gibco - Thermo Fisher
Dulbeccos Modified Eagles Medium (DMEM)/F12	11320082	Gibco - Thermo Fischer
Human Epidermal Growth Factor (hEGF)	PHG0311	Thermo Fisher Scientific
Basic Fibroblast Growth Factor (bFGF)	13256029	Gibco - Thermo Fischer
B-27™ Supplement (50X) minus vitamin A	12587010	Gibco - Thermo Fischer
Costar® 24-well Clear Flat Bottom Ultra-Low Attachment Multiple Well Plates Individually Wrapped S	3473	Corning

MATERIALS TEXT

Humidified, CO2 controlled tissue culture incubator, Cell counter (hemocytometer or automated cell counter), Tissue culture inverted microscope.

1 Prepare Sphere Formation Media (**100 ml**)

Base media is DMEM/F12

1 ml Penicillin and Streptomycin

2 ml B-27

20 µl bFGF

10 µl hEGF

96.97 ml of DMEM/F12.

2 Prepare a single cell suspension from either cell culture or primary tumor tissue. Count cells using a hemocytometer or an automated cell counter.

Citation: Kwangok P Nickel, Austin M. Maas, Randall Kimple (05/15/2020). Isolation of cancer stem cells by sphere formation assay. https://dx.doi.org/10.17504/protocols.io.bdt8i6rw

Note: In this example, a head and neck cancer cell line, UM-SCC47 **SCC071CA.pdf**, is analyzed. The experimental approach can be used to study cells isolated from primary human cancers or with established cancer cell lines.

3 Dilute cells to a final concentration of 2000 cells/ml in Sphere Formation Media.

Note that the final concentration of cells/ml should be optimized to your specific cell line or cancer type.

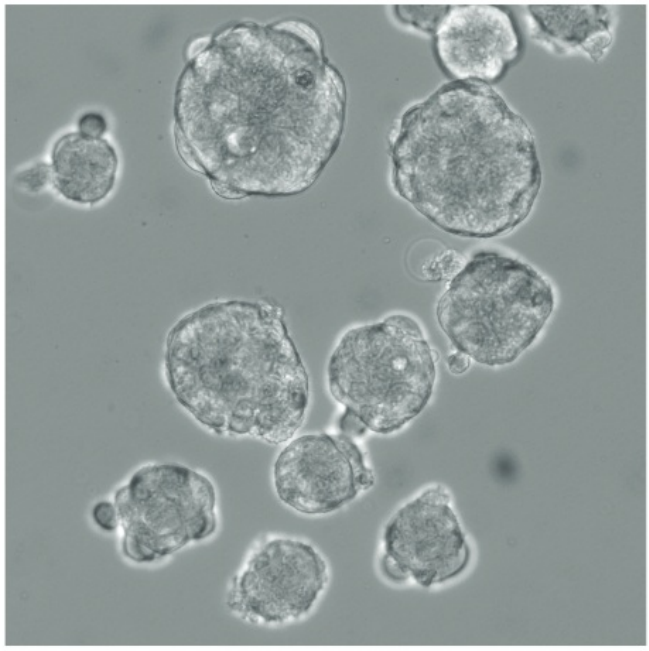
4 Add **250 µl** of cell mix (from Step 3) to each well of a 24-well low attachement plate (final concentration of 500 cells/well).

Note that the optimal number of cells is dependent on tumor type. Use low-attachment plates (see materials) NOT standard tissue culture plates.

5 Place plates into humidified (95% relative humidity) incubator at **37 °C** and 5% CO_2.

6 Check plate daily and add fresh media to keep the wells approximately 20% filled based on evaporation.

7 Check spheres after 2 days and then daily by visualization under a microscope. We use a size of **50 µm** to identify spheres. The time required for sphere formation can vary significantly based on the cell type used. We typically expect approximately 10 spheres per well. In this example, spheres were first identified 3-4 days after plating and a final count was made 7 days after plating the cells.



UM-SCC47 spheres.

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Results

8
- In this assay, the endpoint of interest is sphere formation efficiency (i.e., the percentage of plated cells that form a sphere). This is calculated using the following formula which is calculated for each well.
- Sphere formation efficiency (%) = (number of spheres)/(number of cells seeded) x 100.
- A minimum of 3 biologic replicates should be performed and the average and standard deviation reported.

Table 1. Sphere formation efficiency of UM-SCC47 cells

	well 1	well 2	well 3	Mean	SD
# of spheres	18	18	21		
Efficiency (%)	3.6	3.6	4.2	3.8	0.35

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