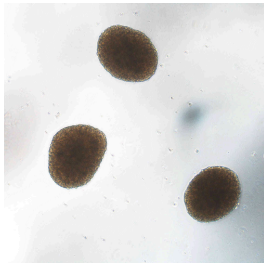
Nov 08, 2022

Generation of glioblastoma spheroid model using an orbital shaker



DOI
dx.doi.org/10.17504/protocols.io.8epv5jz86l1b/v1

WANNAWAT KHOTCHAWAN[1], Chanchao Lorthongpanich[2], Pakpoom Kheolamai[3], Sith Sathornsumetee[4], Surapol Issaragrisil[5]

[1]Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand;
[2]Siriraj Center of Excellence for Stem Cell Research, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand;
[3]Division of Cell Biology, Faculty of Medicine, Thammasat University, Thailand;
[4]Siriraj Center of Research Excellence Management, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand;
[5]Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

W WANNAWAT KHOTCHAWAN
Department of Immunology, Faculty of Medicine Siriraj Hospit...

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.8epv5jz86l1b/v1

Protocol Citation: WANNAWAT KHOTCHAWAN, Chanchao Lorthongpanich, Pakpoom Kheolamai, Sith Sathornsumetee, Surapol Issaragrisil 2022. Generation of glioblastoma spheroid model using an orbital shaker. **protocols.io https://dx.doi.org/10.17504/protocols.io.8epv5jz86l1b/v1**

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: October 13, 2022

Last Modified: November 08, 2022

Protocol Integer ID: 71329

Keywords: Glioblastoma, Orbital shaker, Spheroid, U251-MG, generation of glioblastoma spheroid model, glioblastoma spheroids from the u251 gbm cell line, glioblastoma spheroid model, glioblastoma spheroid, orbital shaker glioblastoma multiforme, tumor behavior in vivo, tumor microenvironment, vivo tumor complexity, u251 cells for experiment, propagating u251 cell, accurate representation of tumor profile, tumor profile, mimicking tumor behavior, effecting tumor growth, spheroid model, tumor growth, u251 gbm cell line, u251 cell line, cell, tumor, 2d system, medication response in vivo, primary brain tumor, orbital shaker, vivo, gbm behavior

Funders Acknowledgements:
Wannawat Khotchawan
Grant ID: Faculty of Medicine Siriraj Hospital, Mahidol University
Chanchao Lorthongpanich
Grant ID: National Research Council of Thailand (N41A640154)

Abstract

Glioblastoma multiforme (GBM) is the most common and aggressive type of primary brain tumor with no effective treatment but a high fatality rate. Thus, a new treatment is still urgently required. The U251 cell line is widely used as a model for investigating GBM characteristics and developing therapeutic treatments. A conventional two-dimensional (2D) culture system is routinely used for maintaining and propagating U251 cells for experiments. However, the 2D system does not correctly mimic the in vivo tumor complexity and tumor microenvironment affected GBM behaviors and responsiveness to drugs treatment. Recent research suggests that three-dimensional (3D) culture methods, such as a spheroid model, could be a better platform for mimicking tumor behavior in vivo. The 3D model can imitate cellular activity, complexity, and providing a much more accurate representation of tumor profiles and medication response in vivo. Here, we provide a simple but yet effective protocol to generate the glioblastoma spheroids from the U251 GBM cell line using an orbital shaker for investigating GBM characteristics and developing therapeutic treatments. This protocol might be applicable to other type of tumors for studying factors effecting tumor growth.

Guidelines

The size and number of spheroid model generated using an orbital shaker might vary when compared to previous techniques for spheroid generation.

Materials

Generation of three-dimensional (3D) glioblastoma spheroids

1. U251 MG cell line human (Sigma Aldrich)
2. DMEM High Glucose; Dulbecco's Modified Eagle Medium (Thermo Fisher Scientific)
3. Fetal bovine serum (FBS)
4. 75-cm2 cell culture flask
5. Sterile seropipettes and pipette tips (1000 µl)
6. Transfer pipettes
7. Trypan blue
8. Hemocytometer
9. Non-treated flat-bottom 6-well plate (Sigma Aldrich)
10. Orbital shaker
11. Tissue culture hood
12. Humidified incubator (37 °C, 5% CO2)

Detection of apoptosis in glioblastoma spheroid model

1. Protein lysis buffer
2. 12% Bis Tris polyacrylamide gels
3. Electrophoresis machine
4. Trans-Blot® SD Semi-Dry Cell (Bio-rad)
5. Rabbit Anti-Caspase-3 antibody (Cell Signaling)
6. Goat anti-rabbit antibody-HRP (Merck)
7. Anti-β-actin-HRP (Cell Signaling)
8. Immobilon Western Chemiluminescent HRP Substrate
9. Biomolecular Imager

Assessment of drug response in glioblastoma spheroid model

1. DMEM High Glucose; Dulbecco's Modified Eagle Medium (Thermo Fisher Scientific)
2. Fetal bovine serum (FBS)
3. Temozolomide (Sigma Aldrich)
4. Orbital shaker
5. Tissue culture hood
6. Humidified incubator (37 °C, 5% CO2)
7. Confocal microscope

Troubleshooting

Safety warnings

- -

Generation of three-dimensional (3D) glioblastoma spheroids

4d

1 Culture the glioblastoma cell line (U251-MG) in the U251-MG media (DMEM-high glucose medium containing 10% fetal bovine serum) on a 75-cm2 cell culture flask for 3–4 days till the cell reach 70–80% confluence before collected for spheroid formation. 5d

2 Perform cell counting using the trypan blue exclusion assay to determine cell number and viability before adjusted concentration to 1× 10^6 cell/ml.

3 Add 2 ml of culture medium (2 × 10^6 viable cells) into 1 well of a non-coated flat-bottom 6-well plate.

4 Set the orbital shaker in a CO_2 incubator which previously been adjusted temperature to 37 °C and 5% CO_2 atmosphere.

5 Place the cell culture plate in step 3 onto an orbital shaker, adjusted the speed to 120 rpm and cultured the cell overnight.

6 The GBM spheres were ready to be collected for the experiment.

Note

If the procedure and technique are followed correctly, a sphere should form after placing the plate overnight on an obital shaker.

Detection of apoptosis in glioblastoma spheroid model

7 The spheroids were collected for protein extraction followed manufacturer's instructions. 2d

8 Briefly, lyse the spheres using cell lysis buffer for protein extraction.

9 Separate the extracted protein by electrophoresis in 12% BisTris polyacrylamide gels and transferred onto PVDF membranes.

10 Perform immunoblotting using primary antibodies against Caspase-3, Cleaved-caspase-3 and horseradish peroxidase conjugated secondary antibodies. Then, image the blot using an appropriate fluorescence scanner.

Assessment of drug response in glioblastoma spheroid model

2d

11 Culture GBM spheroids in U251-MG medium containing 0, 25, 50, and 100 µM of Temozolomide, a standard GBM drug.

12 Place the cell culture plate onto an orbital shaker, adjusted the speed to 120 rpm and cultured the cell for overnight in an incubator which previously been adjusted temperature to 37 °C and 5% CO_2 atmosphere.

13 Observe spheroid morphological changes and number of dead cells after the spheroids being treated with Temozolomide for 24 hours.