

NOV 08, 2022



WORKS FOR ME

Generation of glioblastoma spheroid model using an orbital shaker

DOI

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

<u>WANNAWAT KHOTCHAWAN</u>¹, <u>Chanchao Lorthongpanich</u>², Pakpoom Kheolamai³, Sith Sathornsumetee⁴, Surapol Issaragrisil⁵

- ¹Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand;
- ²Siriraj Center of Excellence for Stem Cell Research, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand;
- ³Division of Cell Biology, Faculty of Medicine, Thammasat University, Thailand;
- ⁴Siriraj Center of Research Excellence Management, Faculty of Medicine Siriraj Hospital, Mahidol University, T hailand:
- ⁵Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Th ailand



COMMENTS 0

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.

DO

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



1

Citation: WANNAWAT KHOTCHAWAN, Chanchao Lorthongpanich, Pakpoom Kheolamai, Sith Sathornsumetee, Surapol Issaragrisil Generation of glioblastoma spheroid model using an orbital shaker 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.

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

FUNDERS ACKNOWLEDGEMENT

4

Wannawat Khotchawan

Grant ID: Faculty of Medicine Siriraj Hospital, Mahidol University

Chanchao Lorthongpanich

Grant ID: National Research Council of Thailand (N41A640154)

KEYWORDS

Glioblastoma, Orbital shaker, Spheroid, U251-MG

LICENSE

This is an open access protocol distributed under the terms of the <u>Creative Commons</u>

<u>Attribution License</u>, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Oct 14, 2022

LAST MODIFIED

Nov 08, 2022

PROTOCOL INTEGER ID

71329

GUIDELINES

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

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

SAFETY WARNINGS

Generation of three-dimensional (3D) glioblastoma spheroids

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.

Perform cell counting using the trypan blue exclusion assay to determine cell number and viability before adjusted concentration to 1× 10⁶ cell/ml.

protocols.io

3

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

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium,

4d

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 $\rm CO_2$ incubator which previously been adjusted temperature to 37 °C and 5% $\rm CO_2$ 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.

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

protocols.io

4

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

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

2d

Assessment of drug response in glioblastoma spheroid model

- Culture GBM spheroids in U251-MG medium containing 0, 25, 50, and 100 μ M of Temozolomide, a standard GBM drug.
- 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% CO₂ atmosphere.
- Observe spheroid morphological changes and number of dead cells after the spheroids being treated with Temozolomide for 24 hours.