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Sub-culturing glioma stem cells (GSCs)

Note: GSC spheres are grown in tissue culture plates that do not promote cell adhesion.

1. Collect GSC spheres from the 10 cm plate using a serological pipet, and place them in a 15 mL conical tube.
2. Spin cells at 800 rpm for 5 min.
3. Aspirate supernatant, and re-suspend cells in 1 mL of accutase (a gentle cell detachment solution).
4. Incubate at 37ºC for 5 min.
5. Add 5 mL PBS to the cells, and gently mix by pipetting until spheres have completely dissociated.
6. Spin cells at 1000 rpm for 5 min.
7. Aspirate supernatant, and resuspend in complete medium.
8. Count cells, and plate for experiments.
9. To subculture, pass ~1×10^6 cells into a new non-adherent 10 cm plate and bring volume to 10 mL with complete medium.
10. Change medium every 2 days by collecting cells in 15 mL conical tubes, pelleting at 800 rpm for 5 min, and re-suspending/plating in fresh medium. Each GSC line grows differently, but most will need to be passaged every 3-5 days. When spheres are too large, they will appear dark in the center and will start aggregating with other spheres.

GSC Medium (500 mL):

486.25 mL	neurobasal medium (Invitrogen 21103049)
5 mL	penicillin/streptromycin (100X)
1.25 mL	L-glutamine (100X)
5 mL	B-27 supplement, minus vitamin A (50X) (Invitrogen 12587010)
2.5 mL	N2 supplement (100x) (Invitrogen 17502048)

Supplement medium with 50 ng/mL human bFGF and 50 ng/mL EGF just prior to plating/subculturing/media changes. Frozen stocks are stored at -20ºC, both at 50 µg/mL. Preparing fresh media with bFGF+EGF prior to use prevents possible degradation of these ligands when stored in stock medium long-term.

Freezing media:

90% complete GSC medium + 10% DMSO