<Sphere formation assay>

1. Preparation

i) Sphere formation medium

<for glioma cells (standard)>

DMEM	25 ml (with antibiotics)
F12 medium	25 ml (with antibiotics)
20 ng/ml of EGF	10 μl of stock (100 μg/ml)
20 ng/ml of bFGF	10 μl of stock (100 μg/ml)
B27 supplement	1 ml

Cells: U251,
NMuMG

<for epithelial tumor cells>

DMEM	25 ml (with antibiotics)
F12 medium	25 ml(with antibiotics)
20 ng/ml of EGF	10 μl of stock (100 μg/ml)
20 ng/ml of bFGF	10 μl of stock (100 μg/ml)
B27 supplement	0.5 ml

Cells: TE13, HaCaT-Ras^{G12V}
154, 234

ii) Dishes

Epithelial tumor cells: Ultra-Low Attachment 10 cm dish (Corning 3262)
Ultra-Low Attachment 6 well plate (Corning 3471)

Glioma cells: Non coated dish (for bacterial culture)

2. Procedure

Cells

↓ Suspend in sphere formation medium and count

↓ Seed in 10 cm (5 x 10^4 cells) or
6 well plate (5 x 10^3 cells)

↓ 5 ~ 14 days (depend on cell type)

↓ Fix with formaldehyde (final 4%)

Microscope OR count visible spheres

If spheres are fused with each other, please consider the addition of methylcellulose (final 1%) to medium.