Mammospheres dissociation and replating:

Serum free mammosphere medium:

(stock)	Final [con]; dilution	25ml	50ml	100ml
B27 (50X)	1X; 50X	0.5ml	1ml	2ml
bFGF (100 µg/ml)	20ng/ml; 5,000X	5ul	10ul	20ul
EGF (10 µg/ml)	20ng/ml; 500X	50ul	100ul	200ul
Gentamycin (10 mg/ml)	100µg/ml; 100X	0.25ml	0.5ml	1ml
AA (100X)	1X; 100X	0.25 ml	0.5ml	1ml
DMEM/F12		23.945ml	47.89ml	95.78ml

Cells are plated on ultra-low attachment plates at a density of 10,000-20,000 viable cells (depend on tumor) /2ml. Cells are fed every 3 days by adding more serum free media. When cultivating for periods longer than 7-10 days, mammospheres are collected, centrifuged at 800 g for 5 min, and resuspended in fresh medium.

For the dissociation of the mammospheres, the pellet is resuspended in 100 ul of 0.05 % trypsin, 0.53 mM EDTA-4Na, and incubated at 37°C for 10 min. Check under the scope to make sure getting the single cells. The suspension is further dissociated mechanically by manual pipetting. Add $HBSS^+$ to kill the trypsin. Then wash w/ HBSS or DMEM/F12 once. After enzymatic and mechanical dissociation, the single cells are re-plated on ultra-low attachment plates in serum free medium. 2,000~10,000 cells/2 ml are plated on secondary culture.

Ultra-low attachment plates: Coring, cat# 3471 (or Fisher cat# 07-200-601)
6 well; case of 24; $175.38
B27 Invitrogen; 17504-044; -20°C; 10ml; $50.50
bFGF Invitrogen; stock (100 µg/ml); -20°C
EGF Invitrogen; stock (10 µg/ml); -20°C
Gentamycin 10 mg/ml (Invitrogen) 4°C
100X Antibiotic-antimycotic (Invitrogen) -20°C
0.05% Trypsin 0.53 mM EDTA Invitrogen 100ml 15305-014