ProMab Biotechnologies, Inc.

Protocol

Disruption and Passaging of Tumorspheres

OVERVIEW

The following protocol is designed for collection and passaging of tumorspheres from a single 6-well plate. The same protocol has been expanded to generate and passage cells from tumorpsheres from four 6-well plates, with no change in viability, or subsequent generation of next-generation tumorspheres.

DISRUPTION PROCEDURE

1. Tumorsphere cultures are incubated for 7-10 days in Cancer Stem Premium™ (catalog no. 20101) media in each of six wells, for a single 6-well plate (tumorspheres, from a suspension of single cells, are visible at approximately 3 days). The majority of tumorspheres at this point will be approximately 50 mm in diameter.
2. The entire culture volume, from all wells (2 mL per well), are transferred to a sterile 50 mL polypropylene centrifuge tube and centrifuged for 10 minutes at 800 rpm.
3. Carefully remove all supernatant, as pellet at this stage may be loose.
4. To the pellet, which from the complete contents of one 6-well plate will be approximately 0.2 mL, is added 0.5 mL of pre-warmed (37°C) Trypsin:EDTA (for 4 x 6-well plates, pellet is approximately 0.5 - 0.75 mL, and 1 mL of pre-warmed (37°C) Trypsin:EDTA is added).
5. Using a sterile P1000 tip (2 mL pipette for 4 x 6-well plates), the tumorspheres are mechanically disrupted. Verifcation of disruption of spheres is performed microscopically.

Should disruption prove difficult, cultures may incubated with trypsin:EDTA for an additional 1 min.

6. Once disruption is complete, add 15 mL of DMEM/10% FBS media to the culture, and centrifuge for 10 minutes at 800 rpm.
7. Remove the media supernatant and resuspend the pellet in 24 mL of Cancer Stem PremiumTM media. Remove an aliquot of cells to perform a viability count, and as soon as it is confirmed that the culture contains a minimum of 90% viable cells, 2 mL are plated per well of a new 6-well plate.
8. Cells are then incubated at 37C/5% C02 for an additional 7-10 days.