SPL Life Sciences – Spheroid Forming Gel User guide

1. Preparation

- 1) Swell the Spheroid forming gel in culture media at 2.5 mg/ml concentration before 24 hours at least
- 2) Check the Spheroid forming gel swelling condition. Centrifuge at 1200 rpm for 5 minutes and discard the supernatant
- 3) Before cell seeding, Add 300 µl of the culture media into each well and insert each
- 4) Incubate 24well plate at 37°C for 15~30 minutes

2. Spheroid culture

- 1) Prepare single cell and remove the media in insert of pre-incubated 24well plate
- 3) Spread 5X10⁴ cells with 20 µl HA solid media to each insert
- 4) Incubate that at 37°C and 5% CO₂ for 5 days

3. Media exchange

- 1) After 1 or 2 days, move the insert to other well and remove culture media in well
- 2) Add new culture media 480~500 µl to well
- 3) Relocate the insert to its original position

4. Spheroid recovery

- 1) Transfer spheroids in insert to a 15ml Conical tube using by 300 μ l of new media
- 2) Repeat step 1) two times
- 3) Transfer all spheroids in insert to a 15ml Conical tube using by 500 µl of new media
- 4) Repeat step 3)
- 5) Wait for at least 10 minutes without moving to sink spheroids, and discard supernatant
- 6) Wash spheroids and use further analysis by PBS or new media

