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℃ for 15~30 minutes

2. Spheroid culture

1) Prepare single cell and remove the media in insert of pre-incubated 24well plate
3) Spread $5X10^4$ cells with 20 µl HA solid media to each insert
4) Incubate that at 37℃ and 5% CO_2 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 µl 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

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