C4-2B

Farnham Lab (150720 version)

1. Thaw the vial containing 1mL of C4-2B cells in a 37^{o}C waterbath. Transfer cells to a 10cm dish containing 9mL of growth medium (RPMI 1640 with 10% FBS and optional addition of penicillin (100 units/mL), streptomycin (100 µg/mL)).
2. Incubate in 5% CO_2 incubator at 37^{o}C overnight.
3. Discard medium carefully, add 10mL of fresh growth medium.
4. Grow cells until about 80% (+/-10%) confluence.
5. Wash cells carefully with warm PBS.
6. Trypsinize cells with 3mL of 0.05% trypsin and 0.02% EDTA at 37^{o}C for 5 min.
7. Neutralize trypsin with 7mL of fresh growth medium.
8. Spin down cells at 470rcf for 5 min at room temperature.
9. Discard supernatant, resuspend cells with 5mL of growth medium, transfer 5mL of cells to a 15cm dish containing 15mL of growth medium growth as new passage.
10. When cells grow to about 80% (+/-10%) confluence, harvest cells by using above steps 5 to 8.
11. Freeze cell stocks by using above steps 5 to 6 with 2x volumes when cells are in a 15cm dish. Discard supernatant, resuspend cells with 3mL of freeze medium (RPMI 1640 with 20% FBS and 10% DMSO), separate into 3 cryo vials, put in foam storage box in -80^{o}C overnight, then remove to liquid nitrogen.