

LNCaP (ATCC, Cat# CRL-1740)

Farnham Lab (150720 version)

Regular culture:

1. Thaw the vial containing 1mL of LNCaP cells in a 37°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% CO₂ incubator at 37°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°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°C overnight, then remove to liquid nitrogen.

DHT Treatment:

1. Grow cells as regular culture until about 80% (+/-10%) confluence.
2. Wash cells carefully with warm PBS.
3. Trypsinize cells with 3mL of 0.05% trypsin and 0.02% EDTA at 37°C for 5 min.
4. Neutralize trypsin with 7mL of fresh growth medium.
5. Spin down cells at 470rcf for 5 min at room temperature.
6. Discard supernatant, resuspend cells 20mL of phenol red free RPMI 1640 with 10% of charcoal stripped FBS, transfer cells to a 15cm dish.
7. Incubate in 5% CO₂ incubator at 37°C for 2 days.
8. After the 2 days, discard medium carefully, add 20mL of fresh phenol red free RPMI 1640 with 10% of charcoal stripped FBS + 10nM DHT or equal volume of ethanol (final ethanol concentration not to exceed 0.01%). Mix DHT/ethanol thoroughly in a 50-mL blue-cap tube before adding to cells.
9. Incubate in 5% CO₂ incubator at 37°C for designed time.