

**SOP:** Propagation of LNCaP  
**Date modified:** 4/20/2009  
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### **Ordering Information**

LNCaP may be ordered from ATCC as a frozen ampoule.

Name: LNCaP, prostate carcinoma  
ATCC #: CRL-1740

### **Notes:**

This is an adherent cell line.

### **Materials List**

1. RPMI 1640 with 2mM L-glutamine (cellgro Cat# 10-040-CM)
2. Fetal Bovine Serum (cellgro Cat# 35-016-CV)
3. T75 & T225 culture flasks
4. Graduated pipets (1, 5, 25mL)
5. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
6. Hemocytometer
7. Micropipet w/ P20 tips
8. Microscope

### **Growth Media for LNCaP**

RPMI-1640 with 2mM L-glutamine  
10% FBS  
1x Pen-Strep

### **Procedure**

#### **A. Receipt of Frozen cells and starting cell cultures.**

- 1) Immediately place frozen cells in liquid nitrogen storage incubator.
- 2) Quickly thaw ampoule in 37°C water bath
- 3) Transfer thawed cells to a T75 flask with 40ml of warm growth media.
- 4) Allow cells to recover over night in 37°C, 5% CO<sub>2</sub> humidified incubator.
- 5) Pour off medium the next day, replace with fresh medium and return to incubator.

#### **B. Sub-culture**

- 1) Propagate cells until density reaches 70-80% confluence.
- 2) Decant medium.
- 3) Wash cells with warm 1X PBS.
- 4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.
- 5) Immediately remove cells and pellet at 500 xg for 3 minutes (4°C)
- 6) Wash cells 2X with 1X PBS.
- 7) Gently re-suspend cell pellet in warm medium.
- 8) Perform 1:3 to 1:6 cell split as needed
- 9) Record each subculture event as a passage

**C. Maintenance**

- 1) Change media the day after seeding and every 72 hours thereafter.  
Use 50ml of media per T225

**D. Harvest**

- 1) Do not use cells that have been passed more than 25 times
- 2) Remove cells from flasks according to protocol described above under 'sub-culturing'
- 3) Examine viability using trypan blue staining.