

## Cell Culture Protocol for U2OS cells

**U2OS (ATCC number HTB-96):** Osteosarcoma;  
Bone sarcoma from the tibia of a human female

Growth medium: DMEM (GIBCO # 11960) + 10% FBS + 2mM L-Glutamine + 100 units/ml penicillin + 100 micro-g/ml streptomycin (GIBCO # 15140-122).

### Protocol for Thawing U2OS Cells:

1. Take out the U2OS stock vial from liquid nitrogen (we freeze at  $2 \times 10^6$  cells per vial) and thaw it at room temperature.
2. Resuspend thawed cells in 10 ml growth media and transfer cells into a 10 sq. cm. tissue culture dish. Cells are grown in a 37°C incubator at 5% CO<sub>2</sub>.

### Protocol for Subculturing of U2OS Cells:

Change medium every 2 to 3 days, and split cultures when they reach 85% confluence ( $1 \times 10^7$  cells/10 sq. cm. dish)

1. Aspirate growth media from the tissue culture dish.
2. Add 5 mls of Trypsin (0.05%) with EDTA solution (GIBCO # 25300) and allow the cells to incubate in the 37°C incubator until cells detach.
3. Add 5 mls of fresh growth media and collect cells in a centrifuge tube.
4. Spin at 1500rpm for 5 minutes.
5. Aspirate supernatant and add fresh growth media
6. Transfer  $1-5 \times 10^5$  viable cells/ml to a new culture vessel and place in a 37°C incubator at 5% CO<sub>2</sub>

Note: We typically split cells at a ratio of 1:4 every 4 to 5 days using the above mentioned seeding conditions.

### Freezing of U2OS Cells:

Cells can be stored as a stock in liquid nitrogen at  $2-5 \times 10^6$  cells/ml in growth medium containing 5% DMSO.