

Cell culture protocol for Ntera-2 cells (ATCC #CRL-1973)

NTERA-2 cl.D1 [NT2/D1] is a pluripotent human testicular embryonic carcinoma cell line. Ntera-2 cells differentiate along neuroectodermal lineages after exposure to retinoic acid (RA). The RA-induced differentiation is characterized by glycolipid changes, appearance of neurons, and induction of homeobox (HOX) gene clusters. The undifferentiated cells have gene expression profiles and chromatin patterns similar to embryonic stem cells.

Comment: Ntera-2 cells will also start to differentiate when plated too sparsely. It is important to maintain cultures at high density to prevent differentiation and maintain the pluripotent state. Seed new flasks at a density of at least 5×10^6 viable cells per 75 sq. cm. flask.

Cell Culture conditions

Ntera-2 growth media: DMEM (GIBCO #11960) with 10% FBS and 2mM L-Glutamine. Pen/Strep can be added, especially for large preps. We purchase 100X Pen/Strep from GIBCO (cat no 15140-122; 100x is 10,000 units/mL penicillin G sodium and 10,000 micro-g/mL streptomycinsulfate) and use at 1X in the cell culture media.

1. Take out the Ntera stock vial from liquid nitrogen (we freeze at 5×10^6 cells per vial) and thaw it in 37 degree water bath. Suspend the washed cells in 5 ml growth media. Attention: When thawing the original stock from ATCC, a small flask has to be used to adjust for the smaller cell number. ATCC cells will NOT grow if plated directly into 75 sq. cm. flask. Cells may also grow more slowly when first purchased.
2. Centrifuge at 1500 rpm for 5 min.
3. Suspend the cells in 15ml growth media and transfer them into a 75 sq. cm. flask. Cells are grown in 37°C incubator at 5% CO₂.
4. Split cultures when they reach ~90% confluency (about 2×10^7 cells per 75 sq. cm. flask). Trypsinize cells and split 1:4 with fresh growth media (about 5×10^6 cells per 75 sq. cm flask). Cells typically reach 90% confluency in 2-3 days, at which point they can be harvested or split back.
5. Cells can be stored as stock in liquid nitrogen at 5 million cells/ml in straight serum (FBS) containing 10% DMSO.