NB4 culture and differentiation

ENCODE members can obtain frozen cell vials of NB4 cells by contacting Sherman Weissman (sherman.weissman@yale.edu). Cells will be made available for the larger research community at the National Cell Culture Center (NCCC; http://www.nccc.com/).

The NB4 cell line, derived from a human promyelocytic leukemia, can be induced to granulocytic maturation by the addition of retinoic acid.[1-3] Alternatively, treatment with tetradecanoyl phorbol acetate (TPA), with or without 1,25-dihydroxyvitamin D3, induces differentiation along a monocytic pathway [2,4,5].

Growth medium: DMEM, high glucose, with 10% heat-inactivated fetal calf serum.
All reagents should be endotoxin free or less than 1EU/ml.
NB4 can also be cultured in RPIM 1640 + 10% H.I.FBS

Frozen stocks: 2 x 10^6cells/ml in 50% DMEM, 40% H.I. FBS, 10% DMSO stored in liquid nitrogen.

To thaw cells, remove vial from liquid nitrogen tank and place immediately into 37^{o}C water bath. When the contents begin to thaw (slurry) gently place cells in a 15ml conical tube. Dropwise add 0.5ml ice cold DMEM (no serum). Gently disperse cells. Wait 1 minute, add 1ml DMEM, gently mix. Wait 1 minute, add 2 ml DMEM. Wait 1 minute, add 4ml DMEM. Centrifuge at 200 x g for 8 minutes, remove supernatant and add 10 ml complete medium to pellet. Transfer to 25 cm^2 tissue culture flask. Incubate in 37^{o}C, 5% CO_2, humidified incubator.

Split cells when the density approaches 0.8 – 1 x 10^6 cells/ml. Maintain cells at 0.2 – 0.8 x 10^6/ml, subculturing every 3-4 days. Cells double in approximately 24 hours, and should be kept below 2 x 10^6/ml. Maintain cells for about 25 passages. Then discard and thaw a fresh stock.

Differentiation:

Granulocytic differentiation:

Culture cells at 2 x 10^5/ml in DMEM + 10% H.I. FBS. Add ATRA (All-trans-retinoic acid, Sigma/Aldrich R2625) to a final concentration of 5-10uM. After 3 days, count cells and keep at 2-5 x 10^5 replace half of the medium and add fresh ATRA. Cells terminally differentiate in 5-7 days as determined by Wright-Giemsa staining.

Monocytic differentiation:

Culture cells at 2 x 10^5/ml in DMEM + 10% H.I. FBS. Add 1,25-dihydroxyvitamin D3 (Sigma/Aldrich D1530) to 200 nM for 8 hours. Wash with PBS, reculture in 200nM TPA (12-O-tetradecanoylphorbol-13-acetate, Sigma/Aldrich P1585) for up to 72 hours. Differentiation is assessed by Wright-Giemsa staining, histochemical testing for non-specific esterase activity, flow cytometry for markers such as CD14.

Reference List

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