Cryopreservation of HD11 cells

Chicken macrophage-like HD11 cells are suspended in complete Roswell Park Memorial Institute (RPMI)-1640 medium with

1. 50% FBS
2. 10% DMSO

and stored at−140◦C.

Thawing and Propagation

a. The chicken macrophage-like cell line HD11, transformed by the avian leukemia virus strain MC29 was maintained in RPMI-1640 containing

1. 25 mM HEPES
2. 300 mg/l L-glutamine
3. 2% chicken serum
4. 8% fetal bovine serum
5. antibiotics (10,000 U/ml penicillin and streptomycin)

at 41°C, 5% CO2, and 95% humidity.

b. The cells should be passaged twice weekly by washing the cells in Dulbecco's phosphate-buffered saline without calcium and magnesium and detaching adherent cells using a 0.25% trypsin/EDTA solution supplemented with phenol red.
c. The cells can be harvested for experiments after 3 to 20 passages – using trypsin/ EDTA
d. Resuspend the cells to a concentration of 200,000 cells/mL in complete RPMI-1640 medium.

Harvesting and experimental use

a. The cells are detached using trypsin/EDTA, harvested and washed with PBS.
b. Aliquots of cell suspensions (2×10^5 cells/ml) are seeded into 96-well culture plates and cultured in RPMI-1640 supplemented with 8% FBS but without antibiotics.
c. The cells were allowed to grow to about 85% confluence during an overnight incubation at 41°C, 5% CO2, and 95% humidity.
d. The cells are exposed to antigens at a fixed concentration of 0.5µL/mL or a concentration gradient of 0.2 – 30 µL/mL to create dose response curves.

e. NO production by the cells can be measured after 48 hours of stimulation by Griess test.

Griess Test

a. Griess reagent:
   3g N-(1-naphtyl) ethylenediamine in 1L 2.5% phosphoric acid - Solution A
   10g sulfanilamide in 1L 2.5% phosphoric acid - Solution B

   Mix sol A and sol B in 1:1 ratio
b. Transfer 50µL of supernatant from stimulated HD11 culture to a 96-well flat bottom cell culture plate.
c. Add 50µL Griess reagent to each well.
   The mixture will turn purple on reaction with nitrite ions in the cell culture supernatant.
d. Read at an optical density of 540 nm

Relative Expression of iNOS and Cytokines

a. HD11 cells are harvested, either 8 h after stimulation or at different time points between 0–48 h, using 200µLPBS+5 mM EDTA
b. Centrifuge at 400×g.
c. Discard the supernatant
d. Resuspend the pellet in RLT buffer and stored at −20◦C until further processing.
e. After thawing, RNA is isolated
f. Prepared cDNA
g. RT-qPCR reactions were performed using 400 nM primers and SYBR Green Master Mix.
h. Changes in gene expression over time upon stimulation are assessed using t=0 as a reference time point and expressed as 2-ΔΔCt-values, according to the Livak method with Ct being the number of cycles before a signal above the threshold (background) level was reached.
i. The results are normalized to gene expression levels of the housekeeping genes 28S and GAPDH.