U937 Culture Protocol

1.Growth Medium

The base medium for Cell line U937 is ATCC-formulated RPMI-1640 Medium(ATCC 30-2001). To complete the growth medium, fetal bovine serum (ATCC 30-2020) will be added to the base medium to a final concentration of 10 %, Penicillin and Streptomycin solution will be added to a final concentration of 1 %.

2.Culture

The cells were seeded in T-75 flasks (catalog #431464) with the cell density between 1 x 10^5 and 2 x 10^6 viable cells/mL and cultured in 5 % carbon dioxide at 37 ℃. The medium will be renewed every 3 to 4 days depending on the cell density.

3.Cryopreservation

The complete growth medium will be supplemented with 5 % (v/v) DMSO and storage at liquid nitrogen vapor phase.

U937 Differentiation Induction Protocol

1.Culture

U937 cells were cultured with RPMI-1640 in 5 % carbon dioxide at 37 ℃.

2.PMA Induction

Cells were seeded at 1 x 10^4 cells/well in 96-well plates in a 100μl volume in the presence of 12.5 nM of phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich) for 48 h to induce differentiation. After washing the PMA-differentiated cells twice, fresh media was added, and the cultures were incubated for an additional 18 h at 37°C in a 5% CO_2 humidified atmosphere before the addition of extracts.