Cytotoxicity Assay

1. U937 cells were cultured with RPMI-1640 in 5 % carbon dioxide at 37 ℃.
2. Cells were seeded at 1x 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.
3. Cells (1x 10^4 cells/well) and peptides were serially diluted to the Minimal Inhibitory Concentration and were cultured in 96-well plates for 72 hours at 37 °C.
4. Finally, the MTS assay was used to evaluate the cytotoxicity of these peptides at 490 nm.
5. Reduction of cell viability by more than 30 % is considered a cytotoxic effect. (ISO 10993-5, 2009)

MTS assay

1. Thaw the CellTiter 96® AQueous One Solution Reagent.
2. Pipet 20µl of CellTiter 96® AQueous One Solution Reagent into each well of the 96-well assay plate containing the samples in 100µl of culture medium.
3. Incubate the plate at 37°C for 1–4 hours in a humidified, 5% CO_2 atmosphere.
4. Record the absorbance at 490 nm using a 96-well plate reader.