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Cytotoxicity assay using LLC-MK2 cell line

luanaborba [1]

[1]Universidade Federal do Rio de Janeiro

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L. P. Borba-Santos

luanaborba

ABSTRACT

Protocol that described the cytotoxicity assay using a mammalian epithelial cell line (LLC-MK2; ATCC CCL-7).

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The protocol that described the cytotoxicity assay using a mammalian epithelial cell line (LLC-MK2; ATCC CCL-7).

1.Confluent monolayers of LLC-MK2 cells were cultivated in flat-bottom 96-well microplates (TPP™) in RPMI 1640 medium[1] supplemented with 2 mM L-glutamine and heat-inactivated 10% fetal bovine serum, and buffered with sodium bicarbonate for 48 hours;

2.The supernatant was gently discarded, samples were washed in sterile PBS, and 100 µl of compounds previously diluted[2] in RPMI 1640 medium were added on confluent monolayers;

3. Cells were treated for 48 h at 37 ºC, in a 5% CO2 atmosphere;

4.The supernatant was gently discarded, samples were washed in sterile PBS, and 150 µl of XTT solution (1 mg/ml XTT[1] and 1mM menadiona[1] in PBS) were added in each well;

5.Microplates were incubated for 2 h at 37°C, in a 5% CO2 atmosphere, in the dark;

6.Microplates were centrifugated at 4000 rpm for 5 min and the supernatant was added into a new microplate;

7. Spectrophotometric readings at 492 nm were performed using a microtiter plate reader[3];

8. The absorbance value for each well was subtracted from the value for the negative controls[4] and inhibition of cell growth (I) relative to positive controls[5] was calculated according to the following equation: I = 100 – (A x 100/C), where A is the absorbance of treated wells, and C is the absorbance of untreated control wells;

9. The concentration of compounds that elicited 50% cytotoxicity (CC50) was estimated by linear regression;

10. The diluent control containing 1% DMSO was included in experiments;

11. Experiments were performed in triplicate in two independent moments.

[1]Sigma Chemical Co., USA.

[2] Stock solutions of compounds in dimethyl sulfoxide (DMSO) at 1 mM were diluted in RPMI 1640 medium supplemented with 2 mM L-glutamine to obtain concentrations of 0.1, 1, 2, 4, 5, 7, 8, 9, and 10 µM.

[3]EMax Plus, Molecular Devices, USA.

[4] Wells without cells containing only RPMI media that were incubated in the same conditions of wells with cells.

[5] Wells with untreated cells.

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