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# Cytotoxicity assay using LLC-MK2 cell line V.2

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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## Cytotoxicity assay using LLC-MK$_2$ cell line

1. The mammalian epithelial cell line LLC-MK$_2$ (ATCC CCL-7) was cultivated in RPMI 1640 medium[1] supplemented with 2 mM L-glutamine and heat-inactivated 10% fetal bovine serum and buffered with sodium bicarbonate at pH 7.5;
2. Aliquots of 100 µl containing 2.5 x $10^5$ cells/ml were added into flat-bottom 96-well microplates (TPP™) and incubated for 48 h at 37 ºC, in a 5% $CO_2$ atmosphere, to obtain confluent monolayers of LLC-MK$_2$ cells;

3.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;

4. Cells were treated for 48 h at 37 ºC, in a 5% $CO_2$ atmosphere;

5.The supernatant was gently discarded and samples were washed in sterile PBS;

6. Cell viability was analyzed by the tetrazolium (XTT) reduction assay and 150 µl of XTT solution (1 mg/ml XTT[1] and 1mM menadiona[1] in PBS) were added in each well;

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

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

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

9. 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;

10. The concentration of compounds that elicited 50% cytotoxicity ($CC_{50}$) was estimated by linear regression;

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

12. 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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