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Cytotoxicity assay using LLC-MK2 cell line V.1

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Abstract

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

Cytotoxicity assay using LLC-MK2 cell line

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 medium¹ 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 diluted² in RPMI 1640 medium were added on confluent monolayers;
3. Cells were treated for 48 h at 37 °C, 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 XTT¹ and 1mM menadione¹ 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 reader³;
8. The absorbance value for each well was subtracted from the value for the negative controls⁴ and inhibition of cell growth (I) relative to positive controls⁵ was calculated according to the following equation: $I = 100 - (A \times 100/C)$, 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.

¹Sigma 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 μ M.

³EMax 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.