

### The study of Antimycin toxicity using renal epithelial cells (HK2) using the Cell IQ®

#### Objective

To measure the toxicity of antimycin using the analysis of cell viability as an end point measurement. As a comparison the Cell IQ results will be compared to conventional procedures using total ATP measurement and uptake of neutral red.

#### Material and methods

##### Cells

HK2 cells,  $2 \times 10^4$  cells/well

Dulbecco's MEM /Nut mix F12 (HAM) (Gibco/Life #31330-038)

ITS: insulin (5  $\mu\text{g/ml}$ ), transferrin (5  $\mu\text{g/ml}$ ) and selenium (5 ng/ml) (# I 1884)

Hydrocortisone (36 ng/ml) (# H 0135)

EGF (10 ng/ml) (# E 1264)

Triiodothyronine (40 ng/ml) (# T 5516)

Antibiotic solution containing 100 units/ml penicillin, 100 g/ml streptomycin, 250 ng/ml amphotericin B

Cells are first incubated overnight in Nunclon 48 well tissue culture plates at 37°C to enable attachment

#### Experimental media

100  $\mu\text{l}$ / well, 3x stock solutions, serum-free

-in glucose-free DMEM (Hypoxia)

control: control: glucose-free DMEM or normal medium

antimycin: antimycin A, 0.5, 1, 5  $\mu\text{M}$  concentrations

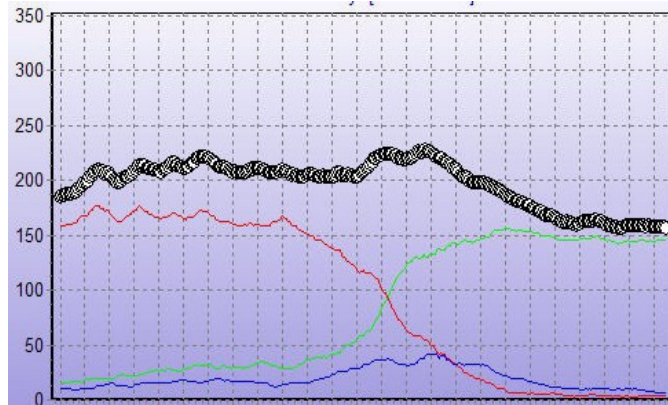
The plates were split to enable one plate to be used for the Cell IQ and two for the total ATP and Neutral red assays respectively.

# APPLICATION NOTE

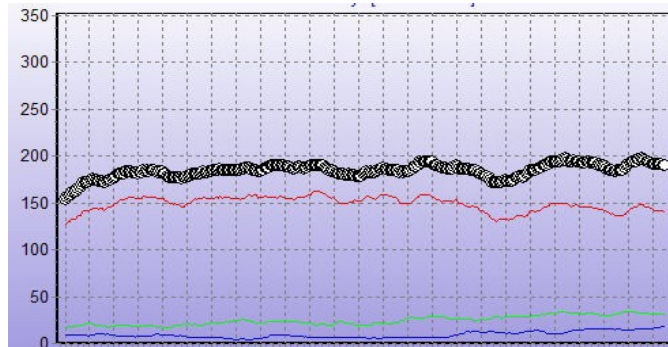
## Results

### *Cell-IQ® Data*

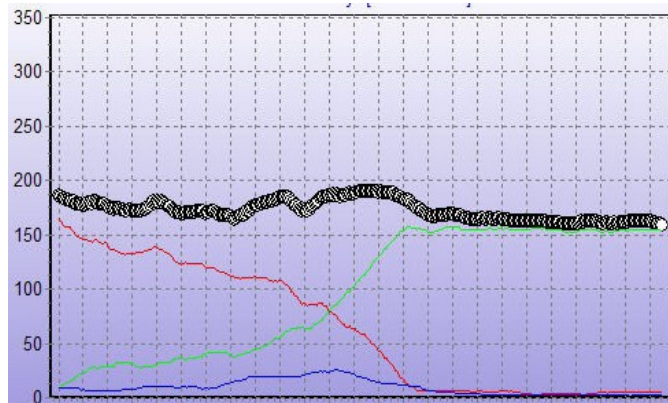
Control



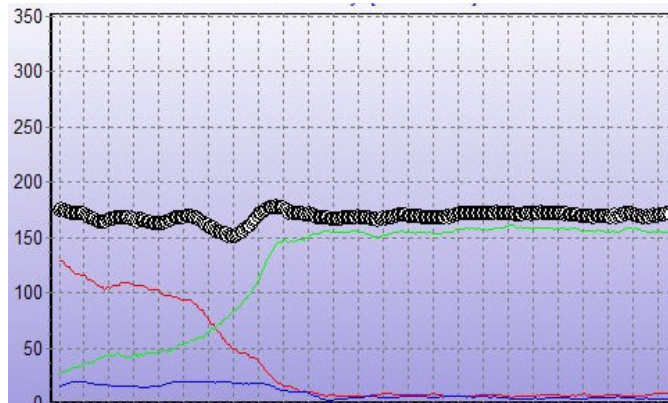
*Antimycin 0.5  $\mu$ M*



*Antimycin 1  $\mu$ M*



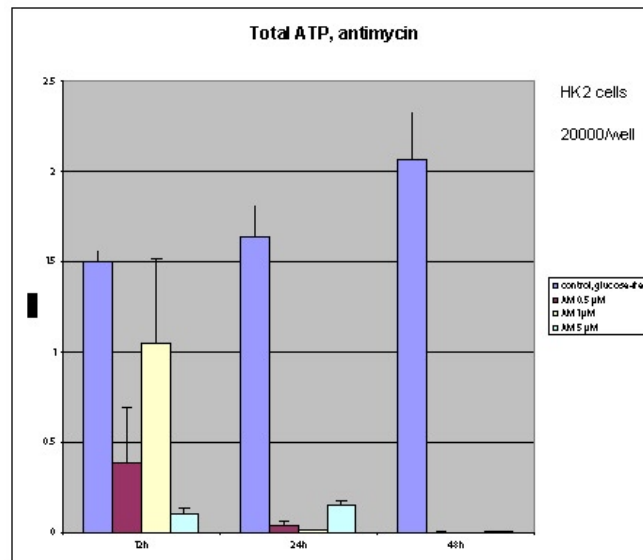
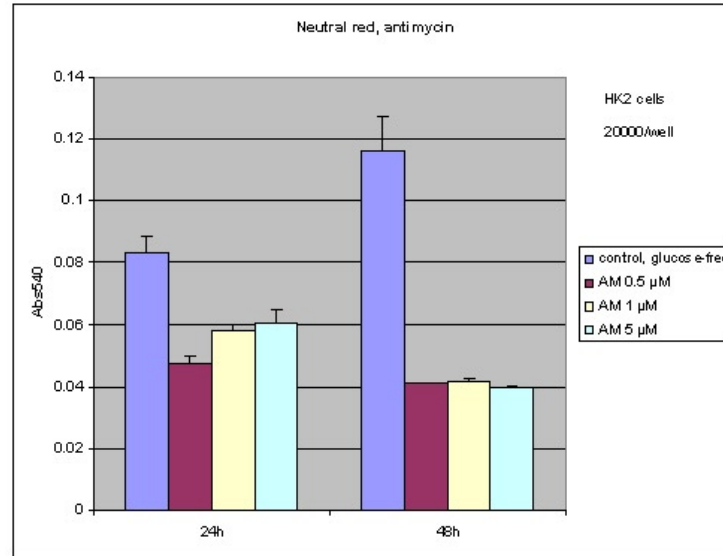
*Antimycin 5  $\mu$ M*



o=Total cell number    red line = stable    green line = dead    blue line = dividing

# APPLICATION NOTE

## Conventional Method Data (possible end points)



## Summary

Selecting a specific end point to measure can be quite challenging. However, Cell-IQ gives a full recording of all changes during the incubation.

Cell-IQ gave comparable results as conventional cell viability tests (ATP and neutral red tests). However, Cell IQ revealed an interesting phenomena namely a delay period of 6-12 hours (depending on antimycin concentration) before acute toxicity was observed, not detected by either end point test. In addition the full incubation by Cell-IQ was recorded allowing re-analysis for additional parameters at anytime in the future.

# APPLICATION NOTE