



**Cell Viability Assessment of Four Test Articles (MTT) in Human Kidney  
Proximal Tubule Cells**

Cyprotex Study Number: CYP1751-R1A

**Prepared For:**

**Test Facility**

Cyprotex US, LLC  
313 Pleasant St.  
Watertown, MA 02472  
USA

**Author**

Shuzhen Qin Ph.D.  
Senior Scientist

Report Date: October 25, 2017

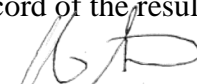
**CONFIDENTIAL**

## Table of Contents

AUTHENTICATION STATEMENT .....	3
1 PURPOSE.....	4
2 STUDY CONDITIONS.....	4
3 EXPERIMENTAL DESIGN .....	4
3.1 Test Articles .....	4
3.2 Cell Viability: experimental conditions .....	4
4 RESULTS AND CONCLUSIONS.....	5
4.1 Cell Viability: Summary .....	5
4.2 Cell Viability: Individual Data.....	5
4.3 Conclusions.....	6

## AUTHENTICATION STATEMENT

I, the undersigned, hereby declare that the work described in this report was performed according to the study protocol and/or standard procedures, and to the best of my knowledge, this report provides a correct record of the results obtained.



\_\_\_\_\_  
Associate Director

October 31, 2017

\_\_\_\_\_  
Date

## 1 PURPOSE

The objective of this study was to assess the cytotoxicity potential of four test articles  
, AB070597 and in Human kidney proximal tubule cells

## 2 STUDY CONDITIONS

This study was performed under non-GLP conditions. All work was performed with appropriate local health regulations and ethical approval.

## 3 EXPERIMENTAL DESIGN

### 3.1 Test Articles

Test Article	Actual MW (FW)
	354.31
	164.20
AB070597	n/a
	226.2

### 3.2 Cell Viability: experimental conditions

Test Article	Test conc.	Reference compound
	500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95 and 0.98 $\mu$ M	CCCP
	500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95 and 0.98 $\mu$ M	CCCP
AB070597	100.00, 50.00, 25.00, 12.50, 6.25, 3.13, 1.56, 0.78, 0.39 and 0.20 $\mu$ g/ml	CCCP
	500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95 and 0.98 $\mu$ M	CCCP

### Experimental Procedure

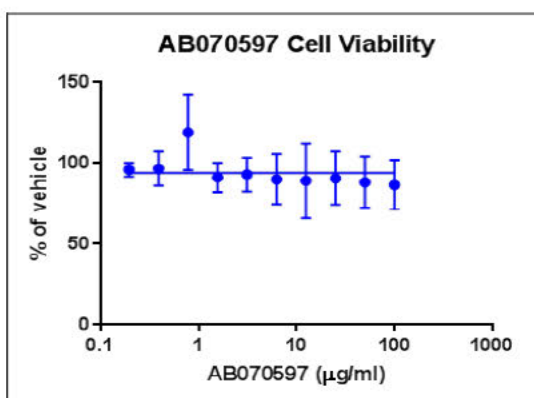
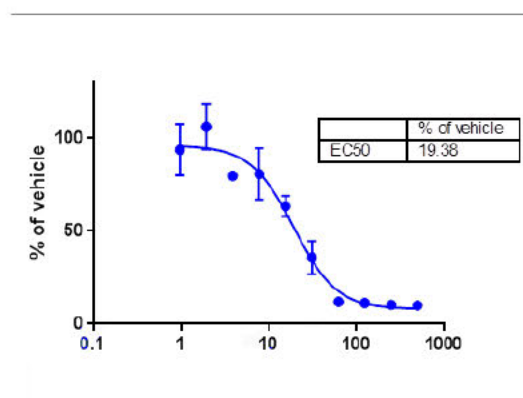
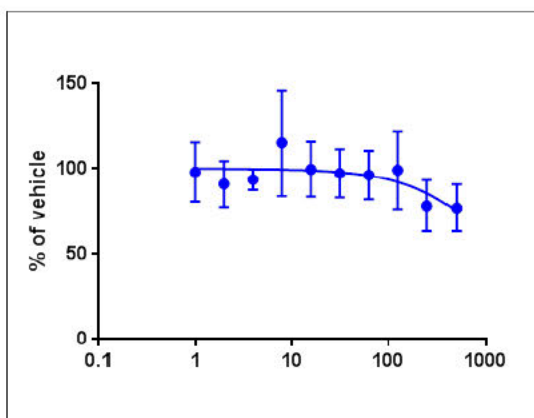
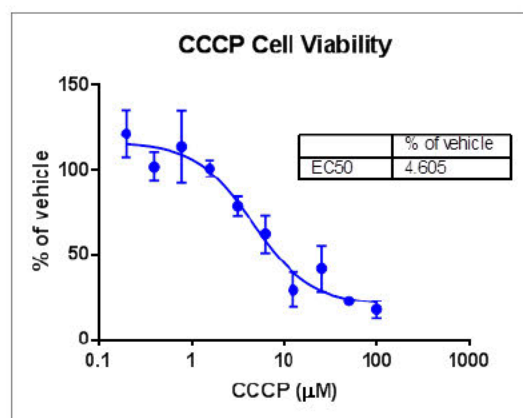
Human kidney proximal tubule cells were plated on 384-well tissue culture treated polystyrene plates at  $1.5 \times 10^4$  cells. After an overnight incubation at 37°C, the cells were dosed with test compounds and controls at a range of concentrations and incubated for 72 hr at 37°C. Cell viability was measured using the Promega CellTiter 96 Non-Radioactive Cell Proliferation Assay (MTT) kit by adding the Dye Solution to each well and incubating for 3 hr at 37°C. After incubation, the Solubilization Solution/Stop Mix was added to each well. Plates were incubated at 37°C for 1 hr, mixed on a plate shaker for 10 min and then absorbance was read at 570nm. The EC<sub>50</sub> was calculated using GraphPad Prism.

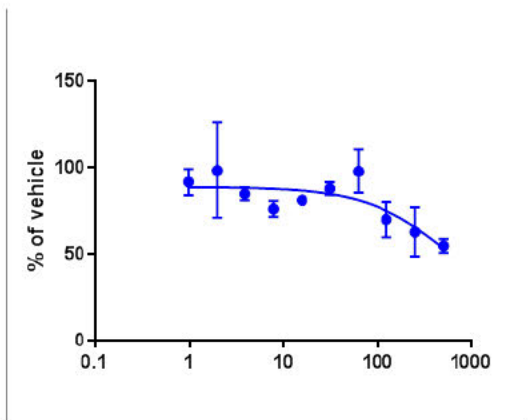
## 4 RESULTS AND CONCLUSIONS

### 4.1 Cell Viability: Summary

Test Article	Cell Viability IC <sub>50</sub>	Comment
CCCP	4.605 $\mu$ M	Control
	> 500 $\mu$ M	Test Article
	19.36 $\mu$ M	Test Article
AB070597	> 500 $\mu$ g/ml	Test Article
	546.9 $\mu$ M	Test Article

### 4.2 Cell Viability: Individual Data





### 4.3 Conclusions

The compounds were tested in Human Kidney Proximal Tubule cells. The positive control compound responded as expected. and AB070597 were not cytotoxic up to their top concentration tested.