Cytotoxicity test report:

Sample: Bacterial cultured extracts

Client:
Enzyme Technology Laboratory

Reported by:
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Cytotoxicity Test Result

April 8, 2011

Dear K. Nuntida,

We have completed the cytotoxicity test for the bacterial cultured extracts as requested. I am enclosing with this letter the test data and results.

The cytotoxicity assay is conformed to the published standard methods (BS-EN30993-5 and ISO10993-5) using L929; a mouse lung connective tissue and human dermal fibroblast cell lines. The cells were exposed to the sample for a period of 24 hours at the concentration of 500 and 100 ug/ml. The results were shown by % survival of cells at each concentration compared to control and IC_{50} values.

The results are limited to the test conditions; no further extrapolation is inferred. BIOTEC will not take any responsibility for any consequences or damages, which may result from this information.

The invoice along with details of the assay and results are appended.

We thank you for your custom; if we can be of further service, please do not hesitate to contact us.

Yours sincerely,

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Cytotoxicity Test Report

April 8, 2011.

Sample preparation

The 18 samples of bacterial cultures were presented to us.

Items	Cells	Broth
1	cH1	bH1
2	cH2	bH2
3	cH3	bH3
4	cH4	bH4
5	cB1	bB1
6	cB3	bB3
7	cC1	bC1
8	cD6	bD6
9	cB1-new	bB1-new

The samples were lyzed in 0.1N NaOH for an hour, and then were adjusted for making stock concentration of 5 and 1 mg/ml. The samples were autoclaved and then filtrated through a 0.2 um filter and were serial diluted in the culture medium of cells giving a concentration of 500 and 100 ug/ml.

Cell culture

The target cells were L929 cell line (mouse lung connective tissue ECACC Cat. No. 85011425) and human dermal fibroblast at passage 6. The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 2mM L-glutamine, 100 unit/ml penicillin and 100 ug/ml streptomycin. The cells were incubated at 37^{o}C in a fully humidified, 5% CO_2 : air atmosphere.

MTT cytotoxicity test, a brief summary of method

This assay was a modified version of conventional direct and indirect contact tests conformed to the published standard methods (BS-EN30993-5 and ISO10993-5). The MTT assay[1] is a tetrazolium-dye based colorimetric microtitration assay. Metabolism-competent cells are able to metabolize the tetrazolium (yellow) to formazan (blue); this color change is measured spectrophotometrically with a plate reader. It is assumed cells that are metabolically deficient will not survive, thus the MTT assay is also an indirect measurement of cell viability. The cells were seeded in a 96-well plate at a density of 3,000 cells/well, and incubated for 48 hours. The sample at various concentrations were added to the cells and incubated for 24 hours. The test samples were removed from the cell cultures and the cells were reincubated for a further 24 hours in fresh medium and then tested with MTT assay.

Briefly, 50 μl of MTT in PBS at 5 mg/ml was added to the medium in each well and the cells were incubated for 4 hours. Medium and MTT were then aspirated from the wells, and formazan solubilized with 200 μL of DMSO and 25 μl of Sorensen's Glycine buffer, pH10.5. The optical density was read with a microplate reader (Molecular Devices) at a wavelength of 570 nm. The average of 8 wells was used to determine the mean of each point. The data were analyzed with the SoftMax Program (Molecular Devices) to determine the IC_{50} for each toxin sample.

[1]Plumb JA, Milroy R, Kaye SB. Effects of the pH dependence of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide-formazan absorption on chemosensitivity determined by a novel tetrazolium based assay. Cancer Res. 1989 **49**: 4435-4440.

Results

The % survival of cells cultured with samples compared to control was summarized below:

Items	Cell lines	Concentration (ug/ml)	Cells	%Survival	Broth	%Survival
1	L929	500	cH1	96±1	bH1	102±3
2		500	cH2	95±6	bH2	98±2
3		500	cH3	102±4	bH3	94±4
4		100	cH4	91±3	bH4	91±3
5		500	cB1	97±2	bB1	65±4
6		500	cB3	99±4	bB3	101±6
7		500	cC1	89±3	bC1	63±2
8		100	cD6	98±1	bD6	97±1
9		500	cB1-new	95±5	bB1-new	70±3
10	Human dermal fibroblast	500	cH1	104±7	bH1	99±2
11		500	cH2	98±2	bH2	93±1
12		500	cH3	93±4	bH3	85±6
13		100	cH4	99±1	bH4	76±2
14		500	cB1	99±3	bB1	73±3
15		500	cB3	97±6	bB3	97±5
16		500	cC1	91±2	bC1	77±2
17		100	cD6	101±3	bD6	89+3
18		500	cB1-new	93±3	bB1-new	96±4

The indication of toxicity has been evaluated in 2 ranges:

a) At % survival >50% will be evaluated for no toxicity
b) At % survival ≤50% will be evaluated for toxicity with IC_{50} value

Conclusion

The cytotoxicity results showed the % survival of L929 and human dermal fibroblast cell lines compared to control. The results showed that all the bacterial cultured extracts were not cytotoxic to both cell lines over the tested concentrations.

DISCLAIMER:

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