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APPENDIX

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX

Growth medium (10% FBS in MEM)

Dissolve 9.4 g Eagle's MEM (Nissui, Japan) powder in 1,000 ml distilled water and sterile by autoclave at 121 °C 15 pound/inch². Add 8 ml of L-glutamine (GIBCO™), 10 ml of 0.14 M HEPES (Research Organic), 100 ml FBS (HyClone®, USA), and adjust pH to 7.4 with 7.5% sodium bicarbonate (Merck).

Maintenance medium (2% FBS in MEM)

Dissolved 9.4 g Eagle's MEM powder in 1,000 ml distilled water and sterile by autoclave at 121 °C 15 pound/inch². Add 8 ml of L-glutamine, 10 ml of 0.14 M HEPES, 20 ml FBS, and adjust pH to 7.4 with 7.5% sodium bicarbonate.

Phosphate Buffered Saline Solution (PBS)

NaCl (Merck, Germany)	8.00	g
KCl (Merck, Germany)	0.20	g
KH ₂ PO ₄ (Merck, Germany)	0.20	g
Na ₂ HPO ₄ •12H ₂ O (Merck, Germany)	1.15	g
distilled water	1,000	ml

This solution is sterilized by autoclave at 121 °C 15 pound/inch².

0.04% Trypsin in PBS

Trypsin (GIBCO™)	0.04	g
EDTA (Fluka)	0.02	g
PBS	100	ml

This solution is sterilized by filtration (0.20 µm filter membrane, Minisart®, Sartorius, Germany).

0.14 M HEPES

N-(2-Hydroxyethyl) piperazine-N'-(2-ethane-sulfonic acid) (HEPES, Research Organic)	3.3362	g
distilled water	100	ml

This solution is sterilized by filtration (0.20 μm filter membrane).

7.5% NaHCO₃

NaHCO ₃ (Merck, Germany)	7.5	g
distilled water	100	ml

This solution is sterilized by autoclave at 121 °C 15 pound/inch².

0.2 % Trypan blue in normal saline

Trypan blue (Fluka)	0.2	g
NaCl	0.85	g
distilled water	100	ml

MTT in PBS

3-(4,5-dimethyl-thiazoyl-2-yl)2,5-diphenyltetrazolium bromide (MTT, USB TM , USA)	100 mg
PBS	50 ml

This solution is sterilized by filtration (0.20 μm filter membrane).

Spectrophotometer for anti-HBsAg activity assay

Lucy 2 ELISA reader, Lucysoft

Spectrophotometer for MTT assay

BIO-RAD Model 550 Microplate reader

Computation of % viability

$$\% \text{ viability} = \left\{ \frac{b - a}{a} \times 100 \right\} + 100$$

a = mean OD of control group

b = mean OD of test sample

If b higher than a, % viability = 100

Computation of % inhibition

$$\% \text{ inhibition} = [(a - b) / a] \times 100$$

a = mean OD of control group

b = mean OD of test sample

If b higher than a, % inhibition = 0

Computation of IC_{50} and CC_{50}

IC_{50} is the concentration of sample which can reduce optical density by 50% compare with control group, calculated with probit/log transformation and regression (Finney, 1971) to produce the equation $Y = a + bX$; when Y = probit value converted from % inhibition, X = log (concentration of sample in $\mu\text{g/ml}$), a = intercept or the distance between X axis and the point where the regression line come across Y axis, and b = slope.

CC_{50} is the concentration of sample which reduce optical density in MTT assay by 50% compare with control group, calculated as same as IC_{50} where as Y = probit value converted from % viability, X = log (concentration of sample in $\mu\text{g/ml}$)

Computation of Selectivity index

Selectivity index (SI) is ratio between CC_{50} and IC_{50} .

Biography

Mister Thaveechai Vachirayonstien was born on April 5, 1979 in Bangkok, Thailand. He received his Bachelor Degree of Science in Pharmacy in 2001 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.



ศูนย์วิทยทรัพยากร
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