

**MICROCARRIER CULTURE OF VERO CELLS FOR
BIOPROCESSING OF BETA-hCG EXPRESSED BY
THE RECOMBINANT VACCINIA VIRUS**

By

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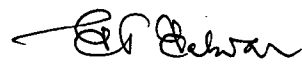
IN MEMORY
OF
MY PARENTS

CERTIFICATE

This is to certify that the thesis entitled MICROCARRIER CULTURE OF VERO CELLS FOR BIOPROCESSING OF β hCG EXPRESSED BY THE RECOMBINANT VACCINIA VIRUS submitted by Asok Mukhopadhyay has been prepared under our supervision in conformity with rules and regulations of Indian Institute of Technology, Delhi.

The research report and results presented in the thesis have not been submitted for any degree in any other Institution.

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ABBREVIATIONS

AMP	: Adenosine monophosphate
ADP	: Adenosine diphosphate
ATP	: Adenosine triphosphate
BMEAA	: Basal medium essential amino acids
BSA	: Bovine serum albumin
c.p.m	: Count per minute
DEPC	: Diethyl pyrocarbonate
DMEM	: Dulbecco's modified eagle medium
DMSO	: Dimethyl sulphoxide
DRB	: 5,6-Dichloro-1- β -D-ribofuranosylbenzimidazole
d	: Day
EEV	: Extracellular enveloped virus
ER	: Endoplasmic reticulum
EDTA	: Ethylene diamine tetraacetic acid
FCS	: Fetal calf serum
Fuc	: Fucose
Gal	: Galactose
GalNAc	: N-acetyl-D-galactosamine
Glc	: Glucose
GlcNAc	: N-acetyl-D-glucosamine
β hCG	: Beta-subunit of human chorionic gonadotropin
h	: Hour
INV	: Intracellular naked virus
MOI	: Multiplicity of infectivity

Man : Mannose
 α -MEM : α -Minimum essential medium
mg : Milligram
Meq : Milliequivalent
NADH : Nicotinamide adenosine dihydrogen
(d)NBCS: Dialyzed new born calf serum
NEAA : Non-essential amino acid
NeuNAc : N-acetylneuraminic acid
ng : Nanogram
PAGE : Polyacrylamide gel electrophoresis
PBS : Phosphate buffered saline
PBST : Phosphate buffered saline + 0.05% Tween-20
PEP : Phosphoenol pyruvate
PK : Pyruvate kinase
PMSF : Phenyl methyl sulfonyl fluoride
p.i : Post-infection
p.f.u : Plaque forming unit
PPO : 2,5-diphenyloxazole
POPOP : 1,4-bis [4-Methyl-5-phenyl-2-oxazolyl] benzene
RER : Rough endoplasmic reticulum
RIA : Radioimmuno assay
SDS : Sodium dodecyl sulphate
TEA : Triethanolamine
TCA : Trichloroacetic acid
 μ g : Microgram

NOMENCLATURES

$AU \cdot mm^2$: Absorbance unit x area of the band in SDS-PAGE

$A_0(t)$: Dimensionless surface area at time "t"

G : Glucose concentration at any time (mM)

G_0 : Initial glucose concentration (mM)

GL : Glutamine concentration at any time (mM)

GL_0 : Initial glutamine concentration (mM)

I_L : Lactate concentration (mM)

I_A : Ammonia concentration (mM)

K : Number of cell hits per bead

k : Attachment rate constant (min^{-1})

M : Ratio of cell-to-bead at $t=0$

N : Number of cells

N_0 : Number of cells during inoculation, $t=0$ (cells/ml)

N_f : Final cell number (cells/ml)

N_F : Number of free cells, time $t=t_1$ (cells/ml)

N_A : Number of attached cells, time $t=t_1$ (cells/ml)

N_{Af} : Number of attached cells, $t=t$ (cells/ml)

N_{F0} : Number of free cells, time $t=0$ (cells/ml)

P : Cellular activity level

P_D : Population doubling

Q_G, \dot{Q}_G : Specific glucose uptake rate ($mM/10^6 \text{ cells.h}$)

- Q_{GL}, \dot{Q}_{GL} : Specific glutamine uptake rate (mM/10⁶ cells.h)
- Q_L, \dot{Q}_L : Specific lactate formation rate (mM/10⁶ cells.h)
- Q_A, \dot{Q}_A : Specific ammonia formation rate (mM/10⁶ cells.h)
- \dot{Q}_{O_2} : Specific oxygen uptake rate (mM/10⁶ cells.h)
- t : Time (minute, hour, day)
- $t_{1/2}$: Half-life (minute, hour)
- $W(K)$: Probability of K number cells hit per microcarrier
- $Y_{N/G}$: Apparent yield of cell based on glucose consumed (cells/mM)
- $Y_{N/GL}$: Apparent yield of cell based on glutamine consumed (cells/mM)
- $Y_{L/G}$: Apparent yield of lactate based on glucose consumed
- $Y_{A/GL}$: Apparent yield of ammonia based on glutamine consumed
- μ : Specific growth rate (h⁻¹)
- $\mu(t)$: Specific growth rate at time "t" (h⁻¹)
- μ_e : Specific growth rate at exponential phase (h⁻¹)

SUBSCRIPTS

- G : Glucose
- GL : Glutamine
- L : Lactate
- A : Ammonia
- O_2 : Molecular oxygen
- e : Exponential

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