PRODUCTION OF NOVEL RECOMBINANT ANTI-PFHRP2 V_{NAR}-G1 PROTEIN USING *ESCHERICHIA COLI* BL21(DE3) EXPRESSION SYSTEM

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by

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A dissertation submitted in the partial fulfillment of the requirements for the degree of Bachelor of Technology (B. Tech) in the field of Bioprocess Technology
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DECLARATION BY AUTHOR

This dissertation is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbol	**Caption**
+	Positive/plus
-	Negative/minus
±	Plus-minus
×	Times
>	More than
<	Less than
%	Percentage
∞	Infinity
^{o}C	Degree Celsius
× g	Relative centrifugal force
K	Potassium
Na	Sodium

Abbreviation	**Caption**
A_{260}/A_{280}	Ratio of absorbance 260 nm to absorbance 280 nm
Aldolase	Fructose 1,6-biphosphate aldolase
ANOVA	Analysis of variance
APS	Ammonium persulphate
bp	Base pair
BSA	Bovine serum albumin
Ca	Calcium

$CaCl_2$	Calcium chloride
CBB	Coomassie Brilliant Blue
cDNA	Complementary DNA
CDRs	Complementary determining regions
CFU/mL	Colony forming unit per millilitre
C_{NAR}	Constant new antigen receptor
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EB	Elution buffer
ECL	Enhanced chemiluminescence
ELISA	Enzyme-linked immunosorbent assay
Fab	Antigen-binding fragment
Fc	Crystallizable fragment
FR	Framework region
Fv	Variable fragment
g	Gram
g/L	Gram per litre
HC	Heavy chains
HCAbs	Heavy-chain-only antibodies
HCl	Hydrochloric acid
His-tag	Histidine-tag
HRP	Horseradish peroxidase
Ig	Immunoglobulin
IgNAR	Immunoglobulin new antigen receptor
IMAC	Immobilized metal affinity

	chromatography
IPTG	Isopropyl-β-D-thiogalactoside
KCl	Potassium chloride
kDa	Kilodalton
KH_2PO_4	Monopotassium phosphate
L	Litre
LB	Luria-Bertani
LC	Light chains
M	Molar
mA	Milliampere
mAbs	Monoclonal antibodies
Mean Sq	Mean of square
MES free acid	2-(*N*-Morpholino)ethanesulfonic acid
MES sodium salt	2-(N-Morpholino)ethanesulfonic acid sodium salt
Mg	Magnesium
mg	Milligram
mg/L	Milligram per litre
mg/mL	Milligram per millilitre
$MgCl_2$	Magnesium chloride
mL	Millilitre
µg	Microgram
µg/mL	Microgram per millilitre
µL	Microlitre
µm	Micrometer

mM	Millimolar
MWCO	Molecular weight cut-off
N	Number of samples
Na_2HPO_4	Disodium phosphate
NaCl	Sodium chloride
NaH_2PO_4	Monosodium phosphate
$NaH_2PO_4 \cdot H_2O$	Sodium dihydrogen phosphate monohydrate
ng/µL	Nanogram per microlitre
nm	Nanometer
OD	Optical density
Omp T	Outer membrane protein T
P. falciparum	*Plasmodium falciparum*
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PfHRP2	*Plasmodium falciparum* histidine-rich protein 2
pLDH	*Plasmodium* lactate dehydrogenase
PMSF	Phenylmethylsulfonyl fluoride
Pr	Probability
R^2	R-squared
RDTs	Rapid diagnostic tests
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	Rounds per minute

RSE	Relative standard error
SB	Super broth
scFv	Single chain variable fragment
sdAbs	Single-domain antibodies
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SOC	Super optimal broth with catabolite repression
Sum Sq	Sum of square
TAE	Tris-acetate-EDTA
TB	Terrific broth
TBS	Tris-buffered saline
TEMED	Tetramethylethylenediamine
TMB	3,3',5,5'-Tetramethylbenzidine
V	Volt
v/v	Volume to volume
VBNC	Viable but non-culturable
V_H	Heavy chain
V_{HH}	Heavy chain single variable domain
V_L	Light chain
V_{NAR}	Variable domain of new antigen receptor
WB	Washing buffer

PENGHASILAN ANTI-PFHRP2 V_{NAR}-G1 PROTEIN REKOMBINAN NOVEL MENGGUNAKAN SISTEM EKSPRESI *ESCHERICHIA COLI* BL21(DE3)

ABSTRAK

Ujian diagnostik segera malaria (RDT) bertindak sebagai imunoassay berasaskan antibodi penting untuk diagnosis segera malaria. Antibodi monoklonal konvensional (mAbs) digunakan secara meluas dalam RDT tetapi ia mudah merosot pada suhu persekitaran tinggi. Oleh itu, V_{NARS} dari ikan yu mungkin merupakan alternatif yang baik untuk mAbs kerana kestabilan haba dan kekuatan gabungan dengan antigen yang lebih tinggi. Dalam kajian ini, anti-PfHRP2 V_{NAR}-G1 protein rekombinan akan dihasilkan dalam sistem ekspresi *E. coli* BL21(DE3) melalui pelbagai langkah seperti pengasingan sel rekombinan, PCR, elektroforesis gel agarosa, pengekstrakan plasmid, transformasi dan ekspresi protein. Selain itu, kesan gabungan suhu dan kepekatan IPTG terhadap kepadatan sel rekombinan BL21(DE3) berdasarkan bacaan serapan dan berat basah sel dianalisis menggunakan perisian R. Berdasarkan analisis statistik ANOVA 2-arah dan regresi berbilang pemboleh ubah, kedua-dua faktor ekspresi mempunyai interaksi gabungan yang sangat signifikan ($p < 0.05$) terhadap bacaan serapan dan berat basah sel. Analisis korelasi antara kepekatan IPTG dan bacaan serapan adalah signifikan ($p < 0.05$) dengan pekali korelasi Pearson yang tinggi (0.9512). Kemunculan anti-PfHRP2 V_{NAR}-G1 protein rekombinan dengan ukuran molekul sekitar 12 kDa dikesan dan disahkan melalui analisis SDS-PAGE dan western blot. Kepekatan protein ditentukan sebagai 0.209 mg/mL dari 0.406 g ekstrak sel kasar. Kesimpulannya, semua objektif dalam kajian ini tercapai dan sdAb rekombinan dari V_{NAR} ikan yu khusus untuk gabungan PfHRP2 berjaya dihasilkan dalam *E. coli* BL21(DE3) sebagai sumber ekspresi.

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ABSTRACT

Malaria rapid diagnostic tests (RDTs) act as important antibody-based immunoassays for prompt malaria diagnosis. Conventional monoclonal antibodies (mAbs) are widely used in RDTs but it can be easily degraded at high ambient temperatures. Hence, the shark V_{NARS} might be good alternatives to mAbs due to its higher thermal stability and binding affinity with antigens. In this study, the recombinant anti-PfHRP2 V_{NAR}-G1 protein was produced in *E. coli* expression system through various steps such as recombinant cell isolation, PCR, agarose gel electrophoresis, plasmid extraction, transformation and protein expression. Besides, the combinatorial effects of temperature and IPTG concentration towards the cell density of recombinant BL21(DE3) based on the absorbance readings and cell wet weights were investigated using software R. Based on the statistical analysis of 2-way ANOVA and multi-variable regression, both expression variables had highly significant combined interactions ($p < 0.05$) towards absorbance readings and cell wet weights. There was significant and strong positive correlation between IPTG concentrations and absorbance readings ($p < 0.05$, $r = 0.9512$). The presence of recombinant anti-PfHRP2 V_{NAR}-G1 protein with a molecular size of about 12 kDa was detected and confirmed through SDS-PAGE and western blot analysis. The protein concentration was determined as 0.209 mg/mL from 0.406 g of crude cell extract. In conclusion, all the objectives in this study were achieved and the recombinant sdAb from shark V_{NAR} specific for PfHRP2 binding was successfully produced in E. coli BL21(DE3) as the expression host.