Supplemental Information

Cloning of PkDBPαII and transfection into mammalian COS-7 cells

Gene amplification and sequencing of PkDBPαII

The PkDBPαII region was amplified by PCR using primers containing a *Bgl*II restriction enzyme cut site, PkDBPαII-F1: 5′-GGCAGATCTGTTATTAATCAAACTTTTCTTC-3′ and PkDBPαII-R1: 5′-AGATCTGTTCAGTTATCGGATTAGAACTG-3′. The amplification reaction was performed using the following thermal cycling condition: 95 °C for 5 min, 30 cycles at 95 °C for 30 s, 55 °C for 45 s and 72 °C for 70 s, followed by a 10-min extension at 72 °C. GoTaq® Flexi DNA Polymerase (Promega, Corp, USA) was used in the PCR. The PCR product of 1027 bp was purified using QIAquick PCR purification kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The purified PCR product was then ligated into cloning vector pGEM-T® (Promega Corp, USA). Each ligation mixture was transformed into One-Shot® TOP10 chemically competent *Escherichia coli* cells (Invitrogen, Carlsbad, CA). Plasmid DNA of recombinant clones harbouring the PkDBPαII fragment was sent to a commercial laboratory (First BASE Laboratories Sdn Bhd, Malaysia) for DNA sequencing. Sequence analysis was performed on two clones for each parasite isolate.

Construction of recombinant plasmids for surface expression on COS-7 cells

The plasmid pDisplay™ (Invitrogen, Carlsbad, CA) is an expression vector designed to target recombinant protein to the surface of mammalian cells. In this study, the fluorescent reporter gene AcGFP (green fluorescent protein from Aequorea coerulescens) was added to the C-terminal of the insert site of pDisplay™ to facilitate direct visualization of the expressed protein. The AcGFP gene was PCR-amplified from the plasmid pAcGFP1-C1 using the forward primer 5′-GTCGACGCCACCATGGTGAGCAAG-3, and reverse primer 5′-GTCGACCTTGTACAGCTCATCCATGCC-3′, which contained a *Sal*I restriction enzyme cut site. The AcGFP gene was then cloned and sequenced. Recombinant pGEM-T® plasmid carrying the PkDBPαII and AcGFP genes was cleaved with *Bgl*II and *Sal*I, respectively, and then cloned into the corresponding sites in the pDisplay™ vector. The plasmid construct, designated as pDisplayAcGFP-PkDBPαII, was purified using a QIAprep Spin Miniprep Kit purification kit (QIAGEN, Hilden, Germany).

Mammalian COS-7 cell transfection

COS-7 (ATCC® CRL-1651™) cells were grown in DMEM-high glucose supplemented with 10% heat inactivated fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine and 1% penicillin–streptomycin at 37 °C in a 5% CO2 incubator. All reagents used were from Gibco™ (Invitrogen, Carlsbad, CA). For transfection, COS-7 cells were plated into six-well culture plates and then transfected with the pDisplayAcGFP-PkDBPαII plasmid DNA (1 µg per well) using 10 µl Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA) in serum-free incomplete DMEM and grown at 37 °C in 5% CO2. After 24 h, the transfection medium was replaced with complete DMEM-high glucose, and the cells were incubated for another 24 h. The transfected COS-7 cells were used in the erythrocyte-binding assay.