Supplementary Information

Table S1. Strains, plasmids, and primers used in this study. The locations of restriction sites in the primer sequences are underlined, and the mutagenic oligonucleotides are in bold.

Strain	Relevant Characteristic(s) or Sequence	Source and/or Reference
E. coli		
BW25113	F−, DE(araD-araB)567, lacZ4787(del)::rrnB-3, LAM−, rph-1, DE(rhaD-rhaB)568, hsdR514	[35]
BL21(DE3)	F− ompT gal dcm lon hsdSB(rB− mB−) λ(DE3 (lacI lacUV5-T7 gene 1 ind1 sam7 nin5))	Novagen
JW1841	*Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), λ^-, *Δzwf-777::*kan, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*	[16]
BL21(DE3)Δ*zwf*::kan^r	F^- ompT gal dcm lon $hsdS_B(r_B^- m_B^-)$ λ(DE3 (lacI lacUV5-T7 gene 1 ind1 sam7 nin5)) *Δzwf-777::*kan	this study
Plasmids		
pJET-1.2	Cloning vector; Amp^R	Thermo Scientific
pET-3a	Expression vector, Amp^R	Novagen
pJg6pd	pJET-1.2 carrying the human *g6pd* gene, Amp^R	this study
pJgK429E	pJET-1.2 carrying the human *g6pd* gene with a K429E mutation in the G6PD protein, Amp^R	this study
pJgR136C	pJET-1.2 carrying the human *g6pd* gene with an R136C mutation in the G6PD protein, Amp^R	this study
pJgR227Q	pJET-1.2 carrying the human *g6pd* gene with an R227Q mutation in the G6PD protein, Amp^R	this study
pJgR393H	pJET-1.2 carrying the human *g6pd* gene with an R393H mutation in the G6PD protein, Amp^R	this study
pETg6pd	pET-3a carrying the human *g6pd* gene, Amp^R	this study
pETgK429E	pET-3a carrying the human *g6pd* gene with a K429E mutation in the G6PD protein, Amp^R	this study
pEtgR136C	pET-3a carrying the human *g6pd* gene with an R136C mutation in the G6PD protein, Amp^R	this study
pETgR227Q	pET-3a carrying the human *g6pd* gene with an R227Q mutation in the G6PD protein, Amp^R	this study
pETgR393H	pET-3a carrying the human *g6pd* gene with an R393H mutation in the G6PD protein, Amp^R	this study
Cloning Oligonucleotides		
Flanking *Nde*I forward	5'-CGACAGC<u>CATATG</u>GCAGAG-3'	[17]
Flanking *Bpu* reverse	5'-TGC<u>GCTGAGC</u>TCAGAGCTT-3'	[17]

Table S1. *Cont.*

Strain	Relevant Characteristic(s) or Sequence	Source and/or Reference
Mutagenesis		
K429E forward	5'-CAGATACA**G**GAACGTGAAGC-3'	this study
K429E reverse	5'-GCTTCACGTTC**C**TGTATCTG-3'	this study
R136C forward	5'-GGCCAAC**T**GCCTCTTCTAC-3'	this study
R136C reverse	5'-GTAGAAG**A**GGCAGTTGGCC-3'	this study
R227Q forward	5'-CTGGAACC**A**GGACAACATCG-3'	this study
R227Q reverse	5'-CGATGTTGTCC**T**GGTTCCAG-3'	this study
R393H forward	5'-GCTGGTGATCC**A**CGTGCAGCCC-3'	this study
R393H reverse	5'-GGGCTGCACG**T**GGATCACCAGC-3'	this study
Oligonucleotides for Sequencing and Verification		
pJET-1.2 forward	5'-CGACTCACTATAGGGAGAGCGGC-3'	Thermo Scientific
pJET-1.2 reverse	5'-AAGAACATCGATTTTCCATGGCAG-3'	Thermo Scientific
Internal G6PD forward	5'-GGCCAACTGCCTCTTCTAC-3'	this study
Internal G6PD reverse	5'-GAGAAGGTCAAGATGTTGAAATG-3'	this study
K1	5'-CAGTCATAGCCGAATAGCCT-3'	[16]
K2	5'-CGGTGCCCTGAATGAACTGC-3'	[16]
−100 bp *zwf* forward	5'-GCTTTTCCCGTAATCGCAC-3'	this study
+100 bp *zwf* reverse	5'-GACTGAAACGCCTGTAACC-3'	this study

Figure S1. Specific activity obtained using the optimal expression conditions in *E. coli* BL21(DE3)Δ*zwf*::kan^r cells is shown. The optimal expression conditions were as follows: for WT G6PD, 0.5 mM IPTG at 25 °C and 18 h expression time; for Yucatan, 0.1 mM IPTG at 25 °C and 18 h expression time; for Valladolid, 0.5 mM IPTG at 25 °C and 18 h expression time; for Mexico City, 0.1 mM IPTG at 25 °C and 18 h expression time; and for Nashville, 0.5 mM IPTG at 37 °C and 6 h of expression time. The G6PD activity after induction with IPTG was indicative of the expression level of the recombinant soluble protein.