PK SOP

Specific activity of the glycolytic enzymes were measured in NAD(P)H/NAD(P)+ linked enzyme assays that were adapted from Teusink *et al*. [1] and measured at 340 nm in 96-well plates (Flat Bottom microplate, Greiner Bio-One, Kremsmünster, Austria) on a spectrophotometer (VarioSkan microplate reader, Thermo Electron Corporation, Waltham, Massachusetts, USA). The same buffer, (20 mM HEPES, 20 mM MgCl, 10 mM KCl and 20 mM NaCl), was used for all assays, with a pH set to 7.17, matching the cytosolic pH of *P. falciparum* D10 [2]. All of the linking enzymes were used at a non-limiting, final concentration of 5 U/mL. All reagents and enzymes were obtained from Sigma-Aldrich, St. Louis, Missouri, USA.

Pyruvate kinase (PK) was measured via the conversion of ADP (0 - 5 mM) and PEP (0 - 5 mM) in the presence of ATP (0 - 5 mM) by linking the reaction to NADH oxidation via LDH and in the presence of PYR (0 - 100 mM) by linking the production of ATP to the reduction of NADP+ (0.8 mM) via G6PDH, HK and GLC (10 mM). Since PK exhibited substrate inhibition, the enzyme rates could not be normalised to maximal specific activity at saturating substrate concentrations. A control rate was determined at 1.25 mM PEP and 0.625 mM ADP.

[1]  Teusink B, Passarge J, Reijenga C, Esgalhado E, van der Weijden C, et al. (2000) Can yeast glycolysis be understood in terms of *in vitro* kinetics of the constituent enzymes? testing biochemistry. Eur J Biochem 267: 5313-5329.

[2]  Wünsch S, Sanchez C, Gekle M, Grosse-Wortmann L, Wiesner J, et al. (1998) Differential stimulation of the Na+/H+ exchanger determines chloroquine uptake in *Plasmodium falciparum*. J Cell Biol 140: 335-345.