HK 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.

Hexokinase (HK) was characterised in the forward direction in terms of glucose (0 -10 mM) and ATP (0 - 10 mM) and inhibition by ADP (0 - 20 mM) by linking the production of G6P to the reduction of NADP+ (0.8 mM) via G6PDH. Product inhibition by G6P (0 - 30 mM) was characterised by linking the production of ADP to the oxidation of NADH (0.8 mM) via LDH and PK in the presence of PEP (2 mM).

[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.