GAPDH 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.

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was assayed with varying substrate concentrations NAD+ (0 - 2.5 mM), GAP (0 - 5 mM) and KH2PO4 (10 mM) and in the reverse direction with NADH (0 - 0.8 mM) and 3PG (0 - 2.5 mM). Since B13PG is not commercially available, 3-phosphoglycerate was used together with PGK and ADP to produce B13PG. Complete and immediate conversion by PGK is assumed to validate the B13PG concentration.

[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.