Activity assay for glucose-6-phosphate dehydrogenase from Caldanaerobacter tengcongensis (CatG6PDH)

Solidzymes protocol 1-3-25

Glucose-6-phosphate dehydrogenase converts G6P to glucose while reducing NAD+ to NADH.

NOTE 1 – This enzyme prefers to use NAD(P)+. This protocol is for specific cases where NAD+ needs to be used.

NOTE 2 – Phosphate is both an activator of this enzyme and a buffer that neutralizes the acidity of NAD+.

Reaction Buffer		
concentration	**reagent**	**amount for 10 mL**
100 mM	phosphate pH 7.0	51.4 mg monobasic 89.1 mg dibasic
20 mM	glucose-6-phosphate	52 mg
5 mM	NAD+	33 mg

NADH standard solution		
concentration	**reagent**	**amount for 1 mL**
200 mM	NADH	133 mg

*NADH stock needs to be kept frozen to avoid oxidation to NAD+.

Prepare the standards

1) Thaw 200 mM NADH, dilute 50X to make a 4 mM stock. Immediately place concentrated stock back into freezer.
2) Put 100 uL of water or phosphate buffer into five wells of a 96-well plate.
3) Mix 100 uL of 4 mM NADH with the first well. Pipette up and down 3-5 times to make 2 mM NADH. Carefully transfer exactly 100 uL from well one to well two and mix to make 1 mM NADH. Continue for all wells to make a standard series from 2 mM – 0.125 mM NADH.
4) Measure the absorbance of the standards at 340 nm and fit to a linear equation.

Activity measurements

1) Prepare a stock solution of 0.05 mg/mL CtG6PDH enzyme.

2) Add 96 uL of Reaction Buffer to wells of a 96-well plate in as many wells as needed for your experiment.
3) Preheat the platereader to 60°C. Once temperature is reached place the plate in the chamber for 5 minutes to equilibrate.
4) Briefly eject the plate and add 4 uL of CtG6PDH stock solution to each well to begin the reaction. Begin measuring the absorbance at 340 nm every 30 seconds for 10 minutes.
5) Export the Mean V from Gen5 platereader software or calculate the average slope in Excel. It may be necessary to discard part of the data at the beginning or end of the ten minute period where the reaction is cold or the detector becomes saturated.
6) Use the NADH standard curve to calculate the increase in molarity per minute. From there calculate the umols of NADH produced per minute per mg of CtG6PDH.

NOTE: The extinction coefficient of NADH is 6220 M^{-1} cm^{-1}. This can be used to calculate the change in molarity of the 100 uL reaction once the pathlength is known.