WCSB, Wageningen University	IBISBA-SOP-WU23
	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: *Acetate kinase protein activity assay*

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changes to prior version:			
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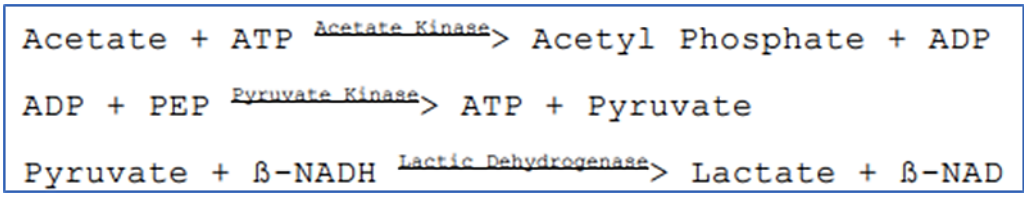
Instruction

Acetate kinase protein activity assay

1. Introduction / Purpose

This protocol describes how to assay the activity of acetate kinase of either cell-free extract or a pure acetate kinase solution. This is what happens during the assay:



Keywords: Acetate kinase – assay

2. Equipment and chemicals

2.1. Equipment

- Platereader (spectrophotometer for 96-wells plates)

2.2. Chemicals

- **A** 100 mM triethanolamine buffer (372g/200ml triethanolamine hydrochloride buffer) Sigma-Aldrich: T1502
- **B** 1 M sodium Acetate solution: Prepare fresh (0.27g/2 ml sodium acetate) Sigma-Aldrich: S2889)
- **C** 91 mM ATP: Prepare fresh (0.05g/ml ATP) Sigma-Aldrich: A6419
- **D** 56 mM PEP: Prepare fresh (0.03 g/ 2ml PEP) Sigma-Aldrich: 10108294001
- **E** 200 mM $MgCl_2$ (2.035 / 50 ml $MgCl_2$) Sigma-Aldrich: M8266
- **F** 6.4 mM β-NADH: Prepare fresh (0.22g/5ml β-NADH (careful: sticky when wet!)) Sigma-Aldrich: 10107735001
- **G** Pyruvate Kinase (PK)/Lactic Dehydrogenase (LDH) enzyme suspension (Used as provided = 600 – 1000 u/ml PK/LDH) Sigma-Aldrich: P0294
- **H** Myokinase enzyme suspension (Used as provided = 2000 – 3000 u/ml myokinase) Sigma-Aldrich: M3003
- **I** Purified acetate kinase (ackA) or CFE 10x diluted in cold **A**

- **Reaction cocktail:** for 10 wells: 1.19 ml **A** + 400 µl **B** + 67 µl **E** + 33 µl **F**

2.3. Other materials

- Cell Free Extract (CFE) of your cells

  S*pecial consumables*

- Clear 96-wells plate

3. Procedures

- Dilute your CFE 10 times in a **different** 96wells plate in **A** (this is **I**, also see above) and store at 4˚C for easy pipetting/repetition of experiment
- Mix carefully in plate: 169 µl **Reagent cocktail** + 3 µl **G** + 1 µl **H**
- Monitor A_{340nm} until constant @ 25 ˚C
- When constant: take plate out of platereader and add 13 µl **C** + 7 µl **D** + 7 µl **I (in blank add A instead of I)**
- Mix immediately
- Record decrease of A_{340nm} for 5 minutes

Calculation of enzyme activity

Use a spreadsheet (Excel) to calculate the enzyme activity:

$$\frac{units}{ml} = \frac{\left[\left(\Delta A340nm \;\; min^{-1}TEST\right) - \;\; \left(\Delta A340nm \;\; min^{-1}Blank\right)\right] * 0.2 * DF}{6.22 * 0.007}$$

0.2 = 200µl reaction volume (total)
DF = dilution factor
0.007 = volume of CFE
6.22 = millimolar extinction coefficient of β-NADH @ 340nm

5. Remarks / troubleshooting

- Timing is critical, so perform all platereader steps next to the platereader.
- Perform each row of your 96 wells plate separately to decrease the amount of time required in between pipetting and analysis
- Stabilization of the platereader can take a relatively long time (>30min). Make sure it is completely stabilized.

6. Biosafety

No biosafety issues are associated with this protocol.

7. Acknowledgements

IBISBA (grant agreement No 730976) and EmPowerPutida (grant agreement No 635536) projects have received funding from the European Union's Horizon 2020 research and innovation programme.

