

Pyruvate:ferredoxin Oxidoreductase (PFR1) Activity Assays Using Methyl Viologen as Artificial Electron Acceptor

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[Abstract] Here we describe the activity measurements of heterologous expressed pyruvate:ferredoxin oxidoreductase (Noth *et al.*, 2013) from *Chlamydomonas reinhardtii*. This enzyme catalyzes the reversible reaction (I) from pyruvate to acetyl CoA and CO₂ generating low potential electrons which are *in vivo* transferred to ferredoxin.



In this assay we use methyl viologen as artificial electron acceptor which turns into dark violet ($\epsilon_{604} = 13.6/\text{Mm/cm}$) (Mayhew, 1978) in its reduced state (Figure 1).

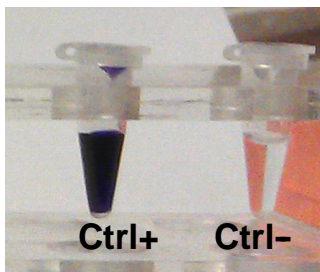


Figure 1. Activity assay using pyruvate, coenzyme A and methyl viologen (Ctrl+). In the absence of pyruvate, no methyl viologen reduction occurs (Ctrl-).

Materials and Reagents

Note: All Reagents are dissolved freshly in an anaerobic tent.

1. Purified pyruvate: ferredoxin oxidoreductase (PFR1)
2. Sodium pyruvate ($\geq 99\%$, Stock 500 mM) (Sigma-Aldrich)
3. Sodium coenzyme A ($\geq 85\%$, Stock 20 mM) (Sigma-Aldrich)
4. Thiamine pyrophosphate ($\geq 95\%$, Stock 250 mM) (Sigma-Aldrich)
5. Methyl viologen (98%, Stock 1 M) (Sigma-Aldrich)
6. Dithioerythriol ($\geq 99\%$, Stock 100 mM) (Carl Roth)

- 0.1 M Tris-HCl buffer (pH 8.0) (see Recipes)

Equipment

- Anaerobic tent (1% H₂, 99% N₂) (Toepffer Lab Systems)
- 96 well plate reader (Beckman Coulter, catalog number: Paradigm1113)
- PC running Multimode analysis software (Beckman Coulter)
- NanoDrop (Paqlab)

Procedure

- Protein concentration of heterologous expressed and purified PFR1 from 2 L of cell culture is measured at A_{280nm} using NanoDrop (ref bio-protocol: [Heterologous Production and Anaerobic Purification of His- and StrepII-tagged Recombinant Proteins](#)).
- All reduction assays are performed under anaerobic atmosphere (1% H₂, 99% N₂) at room temperature.
- The reaction mixture contains 10 mM sodium pyruvate, 2 mM sodium coenzyme A, 5 mM thiamine pyrophosphate, 10 mM methyl viologen and 16 mM dithioerythritol in 0.1 mM Tris-HCl (pH 8).
- To start catalysis a final concentration of 1.4 μM PFR1 is added to the reaction mixture and absorbance (A₆₀₄, 96 well plate reader) can be monitored time resolved every 30 seconds until saturation is reached.
- To determine enzyme activity the molar extinction coefficient $\epsilon_{604} = 13.6 \text{ mM}^{-1} \text{ cm}^{-1}$ (Mayhew, 1978) can be used applying Lambert Beer Law (Eq.I). One unit was defined as the conversion of 1 mol of pyruvate or CoA and the reduction of 2 mol of methyl viologen, respectively, per minute.

$$E_{\lambda} = \epsilon_{\lambda} \cdot c \cdot d \quad (\text{Eq.I})$$

E_λ: extinction

ε_λ: extinction coefficient

c: concentration

d: layer thickness

Recipes

- 0.1 M Tris-HCl buffer (pH 8.0) (1,000 ml)
Mix 12.114 g of Tris base with 800 ml dH₂O

Adjust pH to 8 with HCl
Add ddH₂O to 1,000 ml
Autoclave for 20 minutes at 121 °C
Store at 4 °C

Acknowledgments

Kinetics for enzyme dependent methyl viologen reduction is adapted from Zeikus *et al.* (1977). Research on the pyruvate:ferredoxin oxidoreductase from *C. reinhardtii* was scientifically supported by Anja Hemschemeier and Thomas Happe.

References

1. Mayhew, S. G. (1978). [The redox potential of dithionite and SO₂ from equilibrium reactions with flavodoxins, methyl viologen and hydrogen plus hydrogenase.](#) *Eur J Biochem* 85(2): 535-547.
2. Noth, J., Krawietz, D., Hemschemeier, A. and Happe, T. (2013). [Pyruvate:ferredoxin oxidoreductase is coupled to light-independent hydrogen production in *Chlamydomonas reinhardtii*.](#) *J Biol Chem* 288(6): 4368-4377.
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