

ATPase Assay

Authors: Ilana Kogan, Mohabir Ramjeesingh and Christine E. Bear

**Department of Structural Biology and Biochemistry, The Hospital for Sick
Children and Department of Physiology, University of Toronto,
Ontario, Canada
Tel: 416-813-5981; Fax: 416-813-5028**

e-mail: bear@sickkids.on.ca

Adapted from Kogan et al. (Methods in Molecular Medicine 70, 143-157, 2002)

INTRODUCTION

Our previous studies using purified, reconstituted and phosphorylated CFTR protein show that optimal channel function requires ATP binding and hydrolysis (Bear et al., 1997; Li et al., 1996). Therefore, measurements of the catalytic activity of wild type and mutant CFTR proteins can provide vital information regarding the biochemical, biophysical, and structural properties of CFTR. In the following protocol we describe the methods we use to assay the catalytic activity of purified and reconstituted CFTR. Detailed procedures describing purification of CFTR-His proteins in the presence of the fluorinated surfactant pentadecafluorooctanoic acid (PFO) are published elsewhere (Kogan et al., 2002; Ramjeesingh et al., 1999).

MATERIALS

- Freshly dialyzed CFTR reconstituted into lipid bilayers (30 μ l per reaction)
- 5 mM ATP in a buffer containing: 100 mM Tris base, 200 mM NaCl, 20 mM MgCl₂, pH 7.5 (8 μ l per reaction)
- [α ³²-P] ATP (10 μ Ci/ μ l; Amersham, Oakville, ON)
- Stop solution: 10% SDS, 88% formic acid (v/v)
- PEI cellulose TLC plastic plates, 20 x 20 cm (VWR, Mississauga, ON)
- 5 mM ADP/ATP mixture
- ADP/ATP separation solution: 1 M formic acid, 0.5 M LiCl
- STORM840 Molecular Dynamics PhosphorImager and ImageQuant software package (Molecular Dynamics, Sunnyvale, CA)

METHODS

- The ATPase assay is carried out in a 40- μ l reaction mixture containing the following components:
 - 30 μ l of freshly dialyzed proteoliposomes.
 - 8 μ l of 5 mM ATP in 100 mM Tris base, 200 mM NaCl, 20 mM MgCl₂, pH 7.5.
 - 2 μ l [α ³²-P] ATP (2 μ Ci/reaction).
- Sonicate reactions for 3 sec to distribute components equally inside and outside the proteoliposomes.
- Quick-spin tubes to collect all components of ATPase reaction.
- Incubate reaction at 33°C for 2 hours.
- Stop reaction by addition of 14 μ l of Stop solution. Reactions can be stored at –80°C.
- Pre-spot TLC plates with 1 μ l of 5 mM ADP/ATP mixture and allow to air dry.
- Spot 1 μ l from the ATPase reaction onto the pre-spotted plates and allow to air dry. Place plates into a chamber containing ADP/ATP separation solution and run samples until the solution reaches the top of the plates. Remove plates from chamber and allow to air dry.
- Visualize ADP production in each ATPase reaction by STORM840 Molecular Dynamics PhosphorImager (Figure 1).
- Determine quantity of ATP hydrolyzed by each sample using ImageQuant software.

Figure 1, Kogan et al.

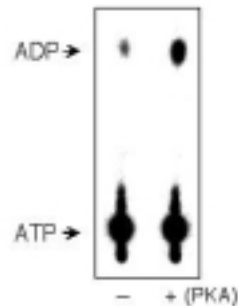


Figure 1 Example of a TLC plate, showing the separation of ADP and ATP by phosphorylated and non-phosphorylated purified and reconstituted wild type CFTR protein

REFERENCES

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