BRIAN FREEMAN'S ATPase ASSAY

ATP hydrolysis is determined by measuring the release of [^{32}P]Pi from [γ^{32}P]ATP according to the protocol of Sadis, S. and Hightower, L.E., *Folded proteins stimulate molecular chaperone Hsc70 ATPase by accelerating ADP/ATP exchange,* Biochemistry (1992).

Reagents:

100 µM ATP (10 mCi [γ-^{32}P]ATP)
0.5 M lithium chloride
0.5 M formic acid

Materials:

polyethyleneimine cellulose thin layer chromatography (PEI-TLC) plates (20x20cm)
PhosphoImager

Buffer C
20 mM HEPES pH 7.2
25 mM KCl
2 mM MgAc
10 mM NH_4SO_2
0.1 mM EDTA

RCMLA	Sigma L-5888
KCl	Fisher BP366-500
[γ^{32}P]ATP	ICN 35020
MgAc	Sigma M-0631
Lithium chloride	Sigma L-9650
Formic acid	Mallinckrodt 2592-1
HEPES	Sigma H-3375
NH_4SO_2	ICN 808 229
PEI-TLC plates	Aldrich Z12,288-2

Procedure:

1. Add 2.5 µg of 70 kDa chaperone (2µM), ATP to final concentration 100µM, and 1µl of 25mCi/ml [γ^{32}P]ATP (10 mCi [γ-^{32}P]ATP) to volume of Buffer C for a total reaction volume of 50µl.
2. At 0 (prior to addition of the 70 kDa chaperone), 5, 10, and 20 min remove and spot a 2µL aliquot onto a polyethyleneimine cellulose thin layer chromatography (PEI-TLC) plate and air dry.
3. The spotted samples can then be resolved utilizing 0.5 M lithium chloride and 0.5 M formic acid.
4. The rates can be calculated utilizing an average [γ^{32}P]ATP hydrolysis rate at each time point (5, 10, 15, and 20 min) from three separate experiments for each sample after the background hydrolysis has been subtracted.
5. Visualize and quantify data by PhosphoImager analysis.

6. The effect of a protein substrate (native □-lactalbumin or RCMLA) on the ATPase rate can be measured in a 1:20 Hsp70:lactalbumin molar ratio prior to incubation at 37°C.

Troubleshooting/Critical Parameters:

Always prepare fresh developing solution. Old solution results in smeared TLC.

References:

Freeman, BC., Myers, MP., Schumacher, R., and Morimoto, RI. Identification of a regulatory motif in Hsp70 that affects ATPase activity, substrate binding and interaction with Hdj1. *EMBO.* **14:**2281-2292 (1995).

Sadis, S. and Hightower, L.E. *Biochemistry*, **31:**9406-9412 (1992).