

Materials List for:

Use of Stopped-Flow Fluorescence and Labeled Nucleotides to Analyze the ATP Turnover Cycle of Kinesins

Jennifer T. Patel¹, Hannah R. Belsham¹, Alexandra J. Rathbone¹, Claire T. Friel¹

¹School of Life Sciences, University of Nottingham

Correspondence to: Claire T. Friel at claire.friel@nottingham.ac.uk

URL: <https://www.jove.com/video/52142>

DOI: [doi:10.3791/52142](https://doi.org/10.3791/52142)

Materials

Name	Company	Catalog Number	Comments
2'/3'-(N-methylanthraniloyl) adenosine-5'triphosphate	Jena Biosciences	# NU-202	mantATP
2'/3'-(N-methylanthraniloyl) adenosine-5'-diphosphate	Jena Biosciences	# NU-201	mantADP
Mg-ATP	Roche	# 10519979001	The disodium salt of ATP is made to a concentration of 100 mM in 100 mM MgCl ₂ . Concentration should be verified by absorption at 260 nm (ε = 15,400 M ⁻¹ cm ⁻¹). Solution stored in aliquots at -20 °C.
Ethylenediaminetetraacetic acid (EDTA)	Fisher Scientific	BP120-500	0.5 M stock solution in water, pH 8.0. HAZARD: powder harmful if inhaled.
Dithiothreitol (DTT)	Melford	MB1015	1 M stock solution in water made fresh for use on the same day. HAZARD: powder harmful if inhaled or in contact with the skin.
NAP-5 column	GE Healthcare	# 17-0853	G-25 sephadex resin gravity-flow gel filtration column
GMPCPP	Jena Biosciences	# NU-405	nonhydrolysable analogue of GTP
Stopped-flow fluorimeter	Applied Photophysics	SX20	A fluorimeter with some plumbing attached, which allows reagents to be rapidly mixed in the observation cuvette. Thereby allowing the kinetics of reactions on timescales of ~0.1 - 100 sec to be monitored.