Introduction

Agilent Bio-HPLC with Wyatt MALS-QELS-RI

- Refrigerated bio-inert system uses non-reactive materials (titanium, gold, ruby, platinum-iridium, ceramic, PTFE, PEEK), has high salt tolerance, and wide pH working range (1-14).

- Applications include preparative, semi-preparative & analytical biological sample and chemical compound purification and characterization, direct enzyme assay applications, GFP-coupled protein expression test.

HPLC system components include:

- Quaternary pump (60 MPa, 0.001—10 mL/min)
- High capacity multi-sampler (vials and plates)
- Peltier-controlled column compartment
- UV-Vis diode array
- Fluorescence spectra and phosphorescence detectors
- Versatile fraction collector in plates or tubes
- Optional in-line MALS-QELS-RI detector modules

Analytical Column Name	Agilent Part #
Bio SEC-3	# 5190-2513
AdvanceBio SEC 300	# PL1580-5301
PL aquagel-OH 30	# PL1120-6830
Agilent Bio WAX NP5	# 5190-2487
Agilent Bio WCX NP5	# 5190-2447
Poroshell 120 EC-C18	# 699975-902
Poroshell 120 EC-C8	# 690970-906T
Poroshell 300 Extend C18	# 670750-902
ZORBAX ODS 70 C18	# 880952-702
ZORBAX Carbohydrate	# 840300-908
AdvanceBio Peptide Map	# 653750-902

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Bio-Inert HPLC

Agilent 1260 Infinity II Bio-Inert HPLC with Multi-angle Static Light Scattering, Quasi-Elastic Light Scattering and Refractive Index

Application Note:

ATP and ADP quantification for NTPase / kinase end point assays

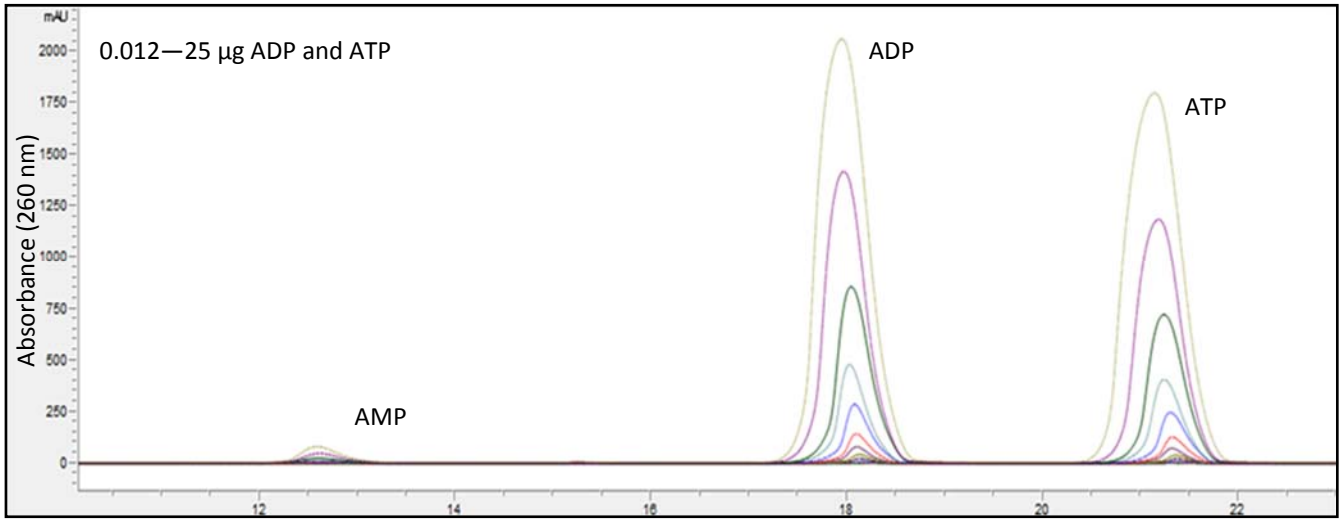
- Quantification of compounds
- Enzyme assays to detect relative amounts of substrate and product

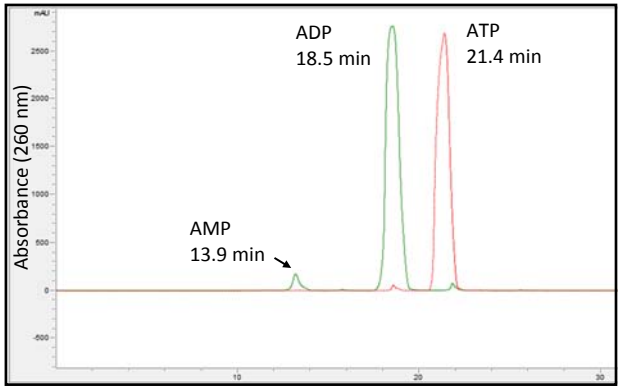
Bio-HPLC with MALS-RI—ATP, ADP Quantification

ATP and ADP quantification

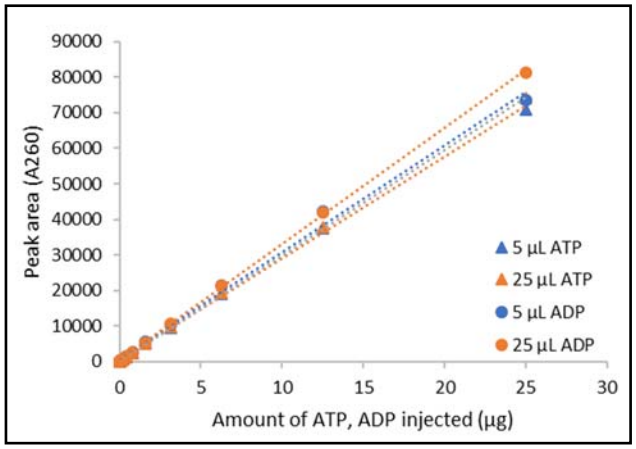
Weak Anion Exchange analytical purification of ATP and ADP

Column: Agilent Bio WAX NP5 (#5190-2487)
Buffers: A—20 mM Tris pH 8.0
B—20 mM Tris pH 8.0, 1 M NaCl
Flow rate: 0.5 mL/min
Elution gradient: 0—50% B in 30 min
Temperature: 5 °C
ATP and ADP Injections : 5 µL (5 µg/uL max), 25 µL (1 µg/µL max)
Sample amount range: 0.012—25 µg ATP/ADP
Detection signal: Abs_{260}





After determining the unique retention times, mixtures of ATP and ADP were serial diluted and injected. Data processing of the peak areas was performed using Agilent's ChemStation instrument software.



Absorbance (260 nm)

Peak	Injection volume	Slope (mAU)	Y-intercept (mAU)	Fit (R^2)
ATP	5 µL	2857	343	**0.999**
	25 µL	2795	683	**0.998**
ADP	5 µL	3009	666	**0.995**
	25 µL	2784	1688	**0.990**

There is a good linear dose response of the peak area versus amount injected.

The linear dose response range is at least 0.012—25 µg of ATP and ADP with an $R^2 > 0.99$.

The quantification of ATP and ADP is accurate when using either 5 µL or 25 µL injection volumes with detection using absorbance at 260 nm. Note that fluorescence (Ex_{266}, Em_{312}) was also used as a detection method with R^2 values similar to that of absorbance detection (data not shown). Note that the optimization of detector sensitivity will extend the linear detection range.

NTPase/kinase End Point Assay Strategy

1. Incubate enzyme and substrate in an appropriate buffer for a standard amount of time and temperature.
2. Stop the reaction and remove the enzyme using a low molecular weight cut-off filtration unit.
3. Inject a set volume of filtrate onto the column to quantify the relative amount of substrate and product.