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Synergistic interaction of glyceraldehydes-3-phosphate dehydrogenase and ArsJ, a novel organoarsenical efflux permease, confers arsenate resistance

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Legends to the Supplemental Figures:

Figure 1S. Multiple alignment of GAPDH sequences. The protein sequence of GAPDH from the *ars* operon of *P. aeruginosa* DK2 (WP_003109848.1) is compared with GAPDH sequences of GapA (WP_003116409.1) and GapB (AFM64157.1) from the chromosome of *P. aeruginosa* DK2, GapA from *E. coli* W3110 (BAA15576.1) and the European rabbit *Oryctolagus cuniculus* (NP_001075722.1). Accession numbers are given in parentheses. The multiple alignment was calculated with CLUSTAL W. Black or gray boxes indicate sequence identity or similarity, respectively.

Figure 2S. Multiple alignment of ArsJ sequences. 2779 ArsJ entries in 2336 bacterial species were identified in the NCBI database, and seven representative sequences were analyzed. The protein sequence of ArsJ from *P. aeruginosa* DK2 (WP_003109849) is compared with ArsJ sequences from *Shewanella putrefaciens* 200 (WP_014610147), *Aeromonas media* (AHX62269), *Marinimicrobium agarilyticum* (WP_027330587), *Bermanella marisrubri* (WP_040298205), *Oceanimonas sp*. GK1 (WP_014290953), *Marinobacter daepoensis* (WP_029654587). Accession numbers are given in parentheses. The multiple

alignment was calculated with CLUSTAL W (Thompson *et al.*, 1994). Black or gray boxes indicate sequence identity or similarity, respectively.

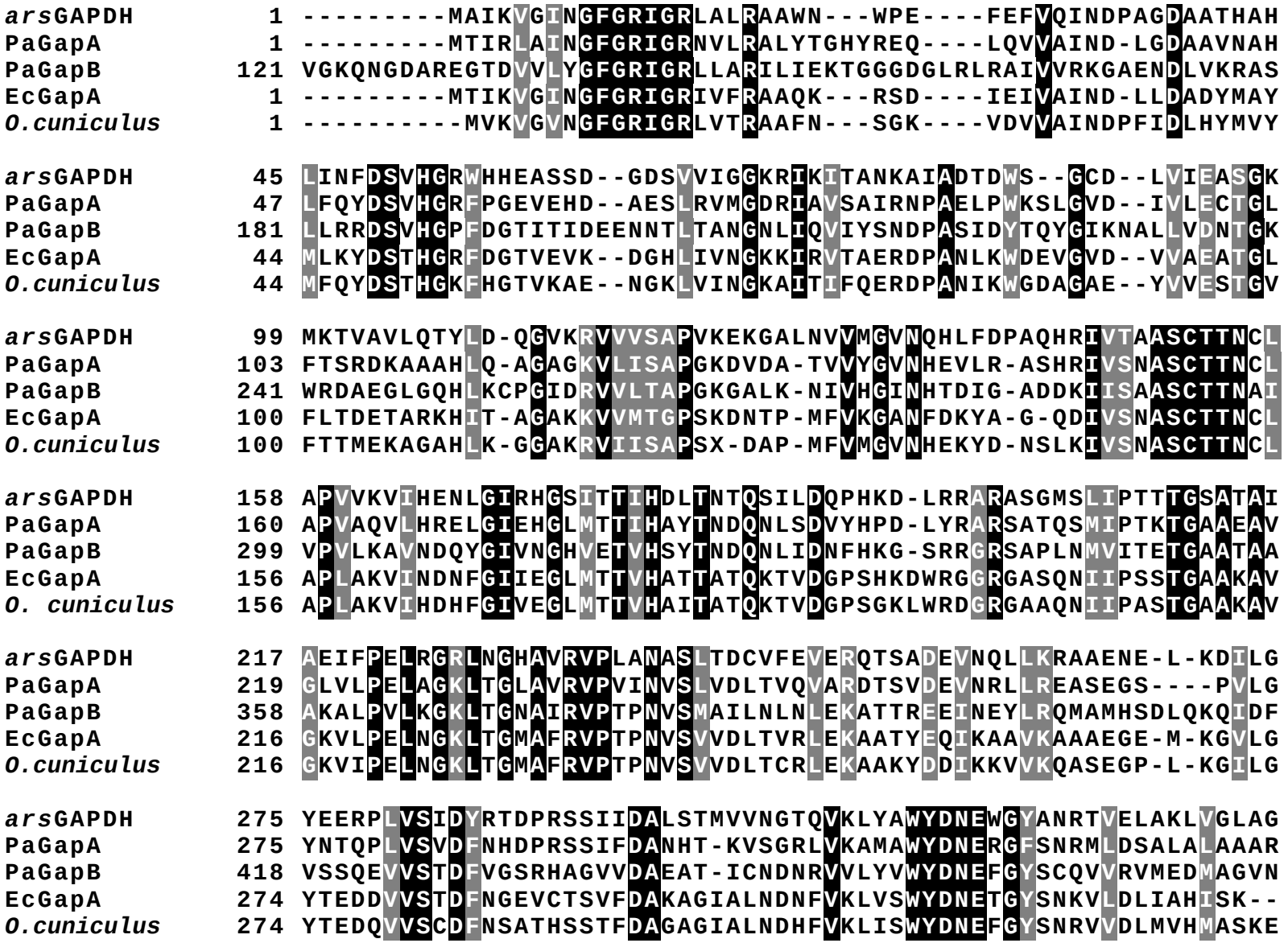
Figure 3S. Arsenate and phosphate as substrates of GAPDH. GAPDH activity was assayed as described in *Experimental procedures* with 1 unit of commercial rabbit GAPDH, 0.6 mM NAD^+, 0.6 mM G3P, 10 mM DTT, with or without arsenate or phosphate, as indicated. Activity was estimated from the absorbance at 340 nm resulting from reduction of NAD^+ to NADH. **A**. Arsenate addition: (□), no As(V); (o), 0.125 mM As(V); (▽), 0.25 mM As(V); GAPDH catalyzed the phosphorolytic-arsenolytic reaction. **B**. Comparison of arsenate and phosphate: (o), no addition; (▽), 50 μM As(V); (□), 50 μM Na_2HPO_4; (◊), 1 mM Na_2HPO_4. **C**. Activity with high phosphate concentrations: (□), no addition; (▽), 3 mM Pi; (o), 10 mM Pi; (◊), 25 mM Pi.

Figure 4S. Substrate requirements for GAPDH activity. GAPDH activity was assayed as described in *Experimental procedures* with the indicated combinations of 1 unit of commercial rabbit GAPDH, 0.6 mM NAD^+, 0.6 mM G3P, 10 mM DTT, or with the indicated arsenicals at 0.1 mM, final concentration. **A**: Specificity for arsenicals: (Δ), none; (▼), As(V); (□), As(III); (◊), MAs(III); (▽), MAs(V); (o), DMAs(V). **B**: Requirement for the components of the GAPDH reaction. (Δ), GAPDH, G3P, NAD^+ and As(V); (◊),G3P, NAD^+ and As(V); (▽), GAPDH, NAD^+ and As(V); (□), GAPDH, G3P and NAD^+; (o),GAPDH, G3P and As(V).

Figure 5S. Arsenate dependence of GAPDH activity. GAPDH activity was assayed as described in *Experimental procedures* with 1 unit of commercial rabbit GAPDH, 0.6 mM NAD^+, 0.6 mM G3P, 10 mM DTT and the indicated concentrations of As(V). A K_m of 0.46 mM As(V) was calculated. Data are the mean ± SE (n = 3).

Figure 6S. GAPDH is not an arsenate reductase. Reduction of As(V) to As(III) was assayed both *in vivo* and *in vitro*. **A**: Arsenate is not reduced in cells of *E. coli* W3110 (parental strain), AW3110 (Δ*arsRBC*) or WC3110 (Δ*arsC*) expressing the *Pseudomonas aeruginosa* DK2 *ars* operon *gapdh* gene (pGAPDH). **B**: Purified rabbit GAPDH reduced almost no As(V) to As(III) after 20 or 40 min of reaction with 1 unit of commercial rabbit GAPDH, 1.2 mM NAD^+, 1.2 mM G3P, 10 mM DTT and 10 μM As(V). Samples were speciated by HPLC using a C18 reverse phase column, and the amount of arsenic in relative counts per second (cps) was estimated by ICP-MS.

Figure 1S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O.cuniculus 1 ----------MVKVGVNGFGRIGRLVTRAAFN---SGK----VDVVAINDPFIDLHYMVY
arsGAPDH 45 LINFDSVHGRWHHEASSD--GDSVVIGGKRIKITANKAIADTDWS--GCD--LVIEASGK
PaGapA 47 LFQYDSVHGRFPGEVEHD--AESLRVMGDRIAVSAIRNPAELPWKSLGVD--IVLECTGL
PaGapB 181 LLRRDSVHGPFDGTITIDEENNTLTANGNLIQVIYSNDPASIDYTQYGIKNALLVDNTGK
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O.cuniculus 44 MFQYDSTHGKFHGTVKAE--NGKLVINGKAITIFQERDPANIKWGDAGAE--YVVESTGV
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Figure 2S

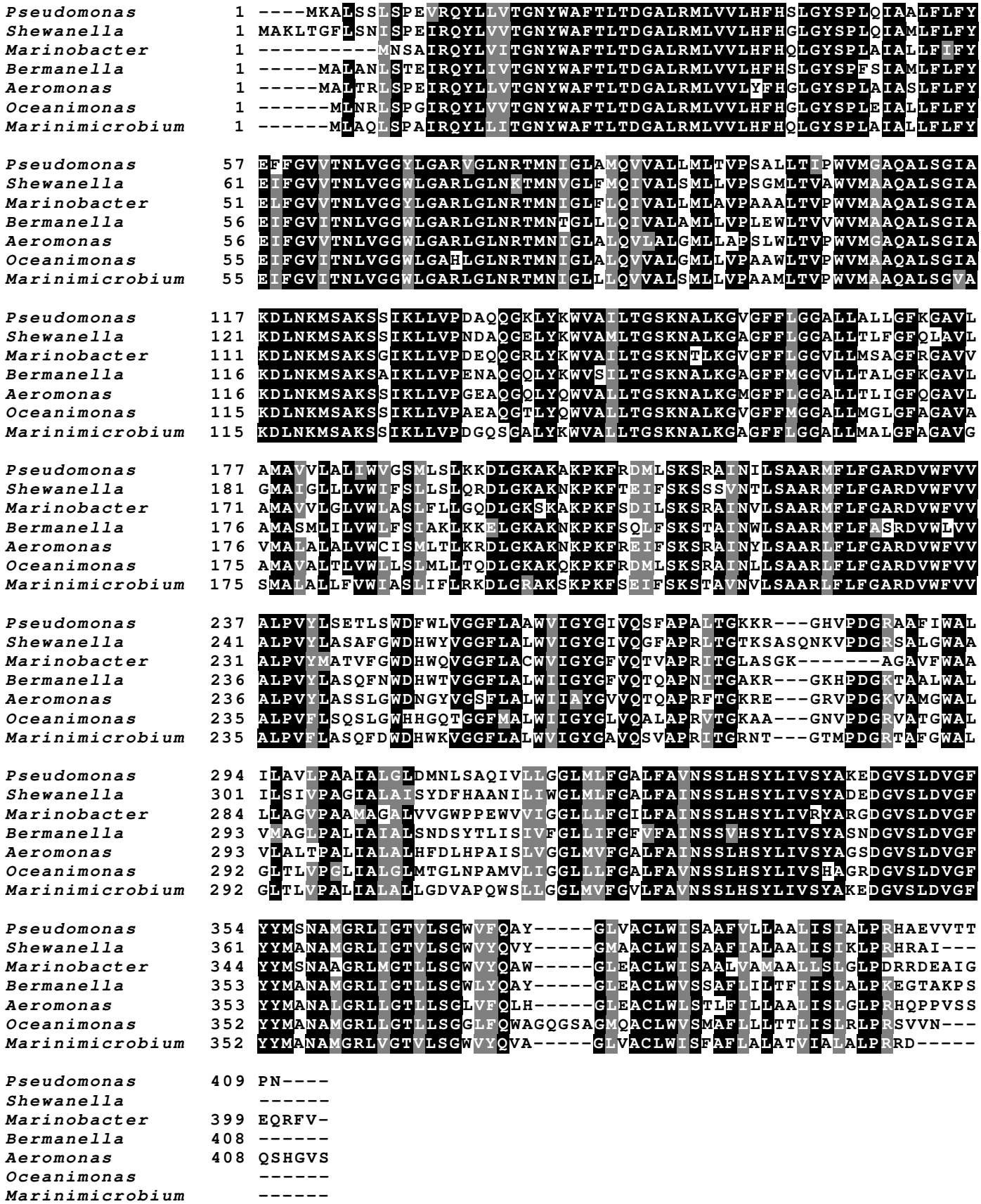
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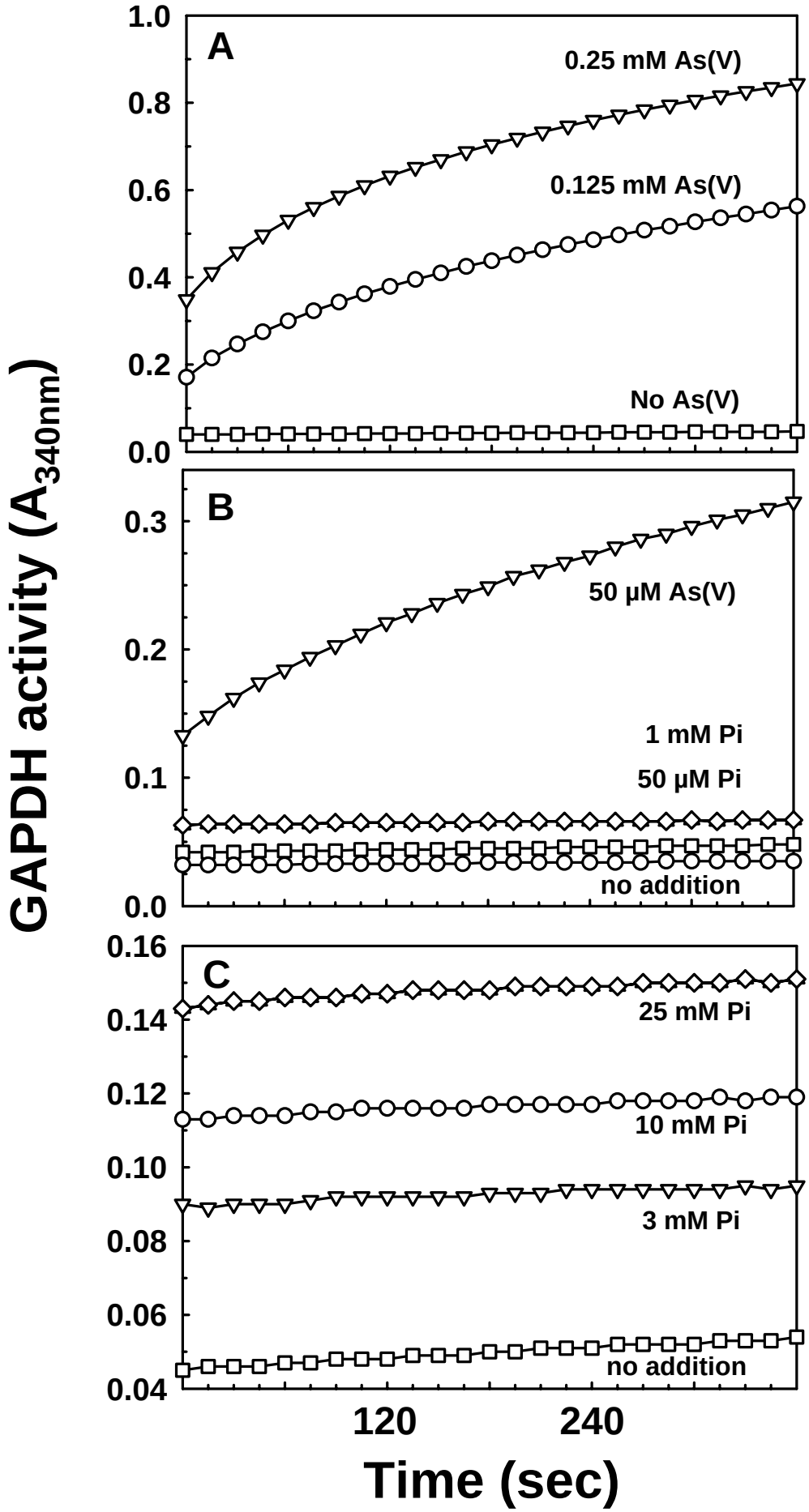
Figure 3S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0.25 mM As(V)
0.125 mM As(V)
No As(V)
B
50 µM As(V)
1 mM Pi
50 µM Pi
no addition
C
25 mM Pi
10 mM Pi
3 mM Pi
no addition
GAPDH activity (A340nm)
Time (sec)
1.0
0.8
0.6
0.4
0.2
0.0
0.3
0.2
0.1
0.0
0.16
0.14
0.12
0.10
0.08
0.06
0.04
120
240

Figure 4S

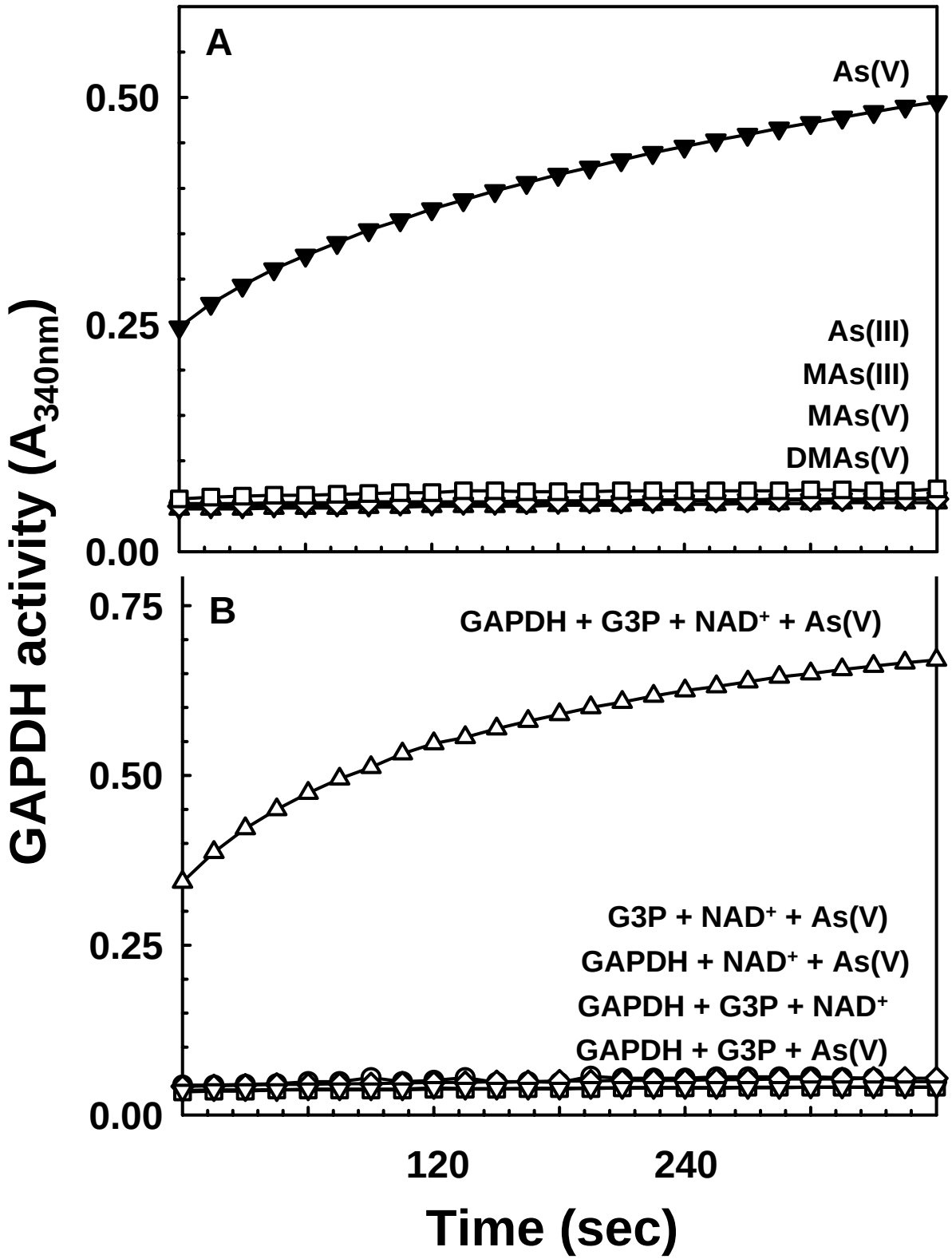

A
As(V)
As(III)
MAs(III)
MAs(V)
DMAs(V)
0.50
0.25
0.00
B
GAPDH + G3P + NAD+ + As(V)
G3P + NAD+ + As(V)
GAPDH + NAD+ + As(V)
GAPDH + G3P + NAD+
GAPDH + G3P + As(V)
0.75
0.50
0.25
0.00
GAPDH activity (A340nm)
120
240
Time (sec)

Figure 5S

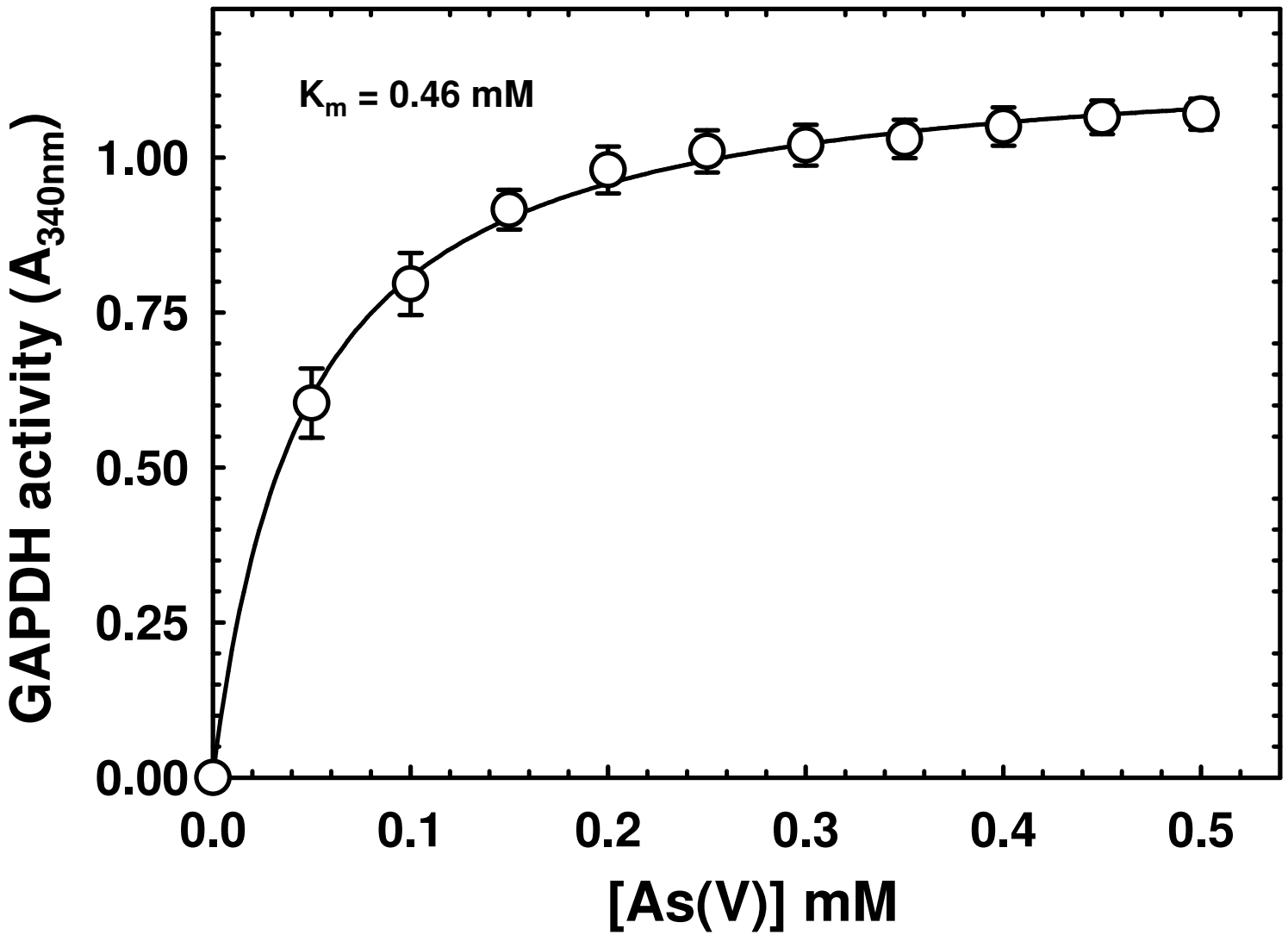
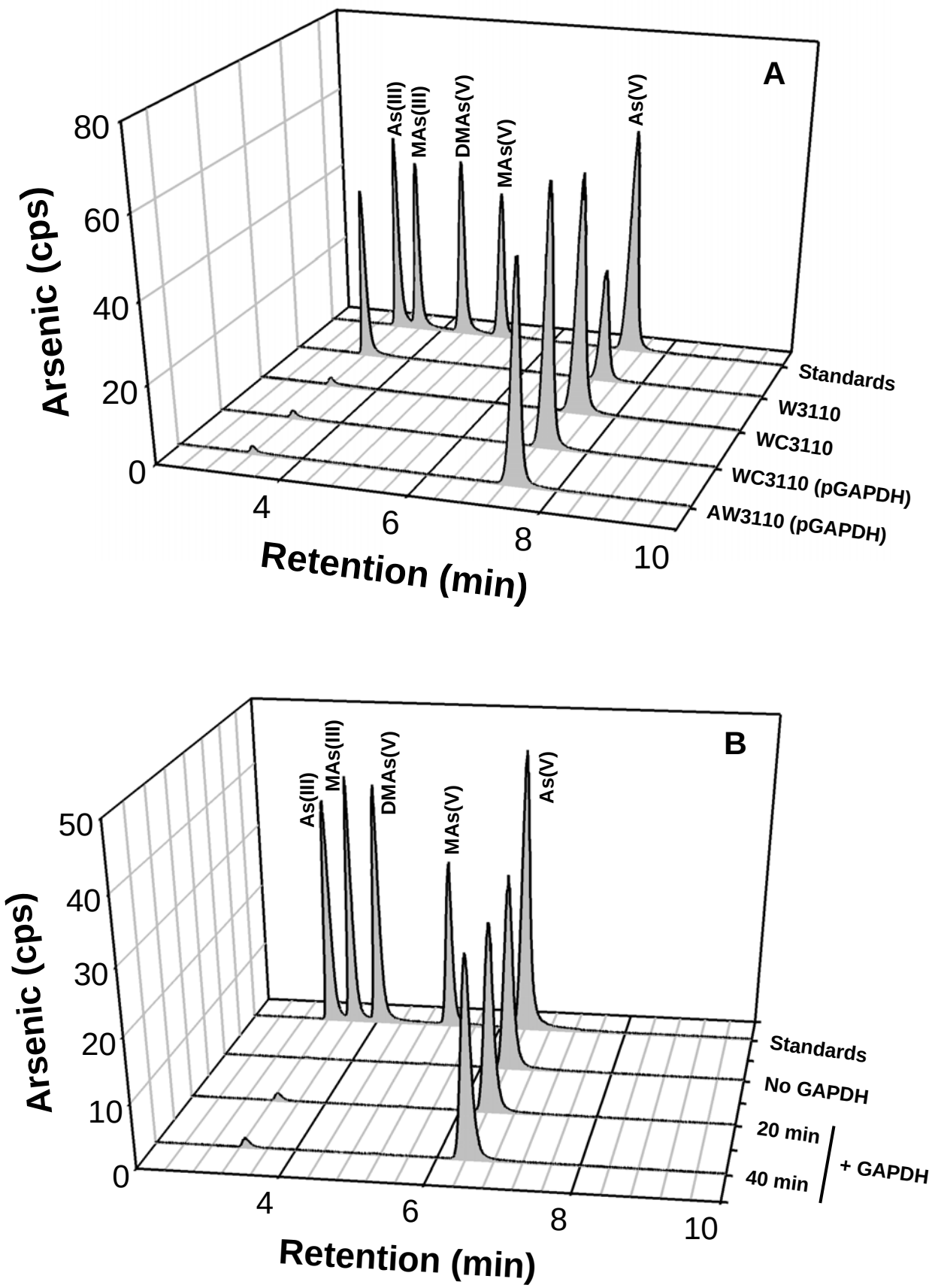

K_m = 0.46 mM
GAPDH activity (A_{340nm})
1.00
0.75
0.50
0.25
0.00
0.0
0.1
0.2
0.3
0.4
0.5
[As(V)] mM

Figure 6S


A
As(III)
MAs(III)
DMAs(V)
MAs(V)
As(V)
Arsenic (cps)
80
60
40
20
0
4
6
8
10
Retention (min)
Standards
W3110
WC3110
WC3110 (pGAPDH)
AW3110 (pGAPDH)
B
As(III)
MAs(III)
DMAs(V)
MAs(V)
As(V)
Arsenic (cps)
50
40
30
20
10
0
4
6
8
10
Retention (min)
Standards
No GAPDH
20 min
40 min
+ GAPDH