Product Data Sheet

Recombinant Multidrug Efflux Pump Subunit AcrB

Information

PRODUCT DETAILS	
Compound Name	Multidrug Efflux Pump subunit AcrB
Catalog Numbers	41-T401A-10UG 41-T401A-50UG 41-T401A-BULK
Class	Multidrug efflux pump
Sequence	Full-length, wildtype sequence, with a C-terminus 6xHis-tag: MPNFFIDRPIFAWVIAIIIMLAGGLAILKLPVAQYPTIAPPAVTISASYPGADAK TVQDTVTQVIEQNMNGIDNLMYMSSNSDSTGTVQITLTFESGTDADIAQVQ VQNKLQLAMPLLPQEVQQQGVSVEKSSSSFLMVVGVINTDGTMTQEDISD YVAANMKDAISRTSGVGDVQLFGSQYAMRIWMNPNELNKFQLTPVDVITAI KAQNAQVAAGQLGGTPPVKGQQLNASIIAQTRLTSTEEFGKILLKVNQDGS RVLLRDVAKIELGGENYDIIAEFNGQPASGLGIKLATGANALDTAAAIRAELA KMEPFFPSGLKIVYPYDTTPFVKISIHEVVKTLVEAIILVFLVMYLFLQNFRAT LIPTIAVPVVLLGTFAVLAAFGFSINTLTMFGMVLAIGLLVDDAIVVVENVERV MAEEGLPPKEATRKSMGQIQGALVGIAMVLSAVFVPMAFFGGSTGAIYRQF SITIVSAMALSVLVALILTPALCATMLKPIAKGDHGEGKKGFFGWFNRMFEK STHHYTDSVGGILRSTGRYLVLYLIIVVGMAYLFVRLPSSFLPDEDQGVFMT MVQLPAGATQERTQKVLNEVTHYYLTKEKNNVESVFAVNGFGFAGRGQNT GIAFVSLKDWADRPGEENKVEAITMRATRAFSQIKDAMVFAFNLPAIVELGT ATGFDFELIDQAGLGHEKLTQARNQLLAEAAKHPDMLTSVRPNGLEDTPQF KIDIDQEKAQALGVSINDINTTLGAAWGGSYVNDFIDRGRVKKVYVMSEAK YRMLPDDIGDWYVRAADGQMVPFSAFSSSRWEYGSPRLERYNGLPSMEI LGQAAPGKSTGEAMELMEQLASKLPTGVGYDWTGMSYQERLSGNQAPS LYAISLIVVFLCLAALYESWSIPFSVMLVVPLGVIGALLAATFRGLTNDVYFQV GLLTTIGLSAKNAILIVEFAKDLMDKEGKGLIEATLDAVRMRLRPILMTSLAFI LGVMPLVISTGAGSGAQNAVGTGVMGGMVTATVLAIFFVPVFFVVVRRRF SRKNEDIEHSHTVDHHLEHHHHHH
Affinity Tag	His-tag (C-terminal)
Origin	*Escherichia coli* (strain K12)
Theor. MW	114.6 kDa
Accession #	P31224 (UniProt)
Purification	Immobilized Metal Affinity Chromatography
Purity	>95%
Activity	Confirmed by Ligand binding assay

PRODUCT DETAILS	
Sample Buffer	25 mM Na_2HPO_4/NaH_2PO_4 pH 8.0, 150 mM NaCl, 0.01% DDM
Available Quantity	From 10 µg up to mg scale
Shipment Temperature	Dry ice
Storage Conditions	Store at -80°C

Quality Controls

Purity

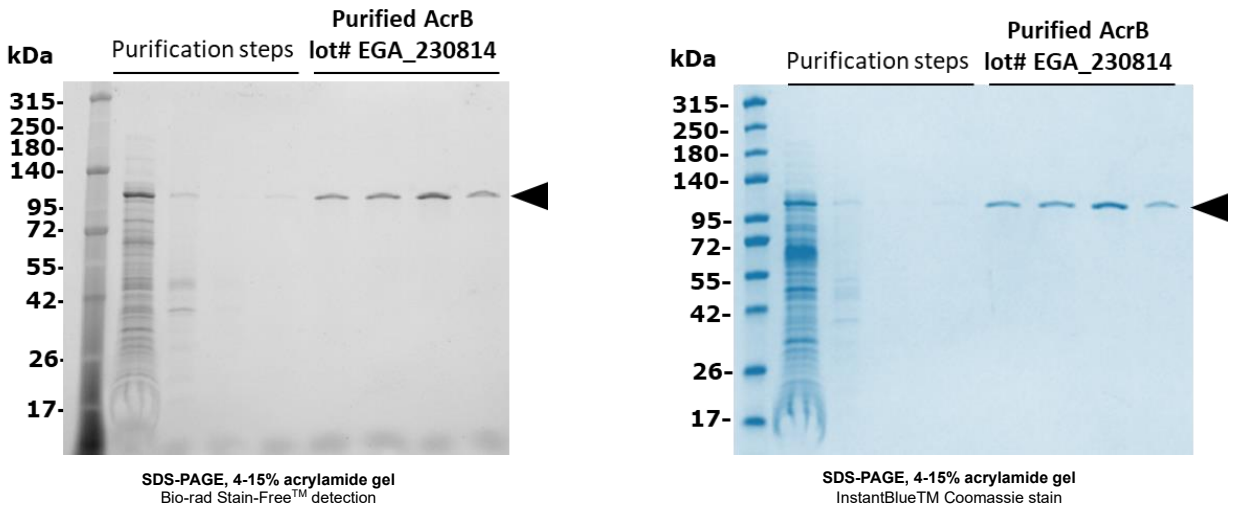


Figure 1: SDS-PAGE, Stain-Free™ detection (left) and SDS-PAGE, Coomassie stain (right).
Purified AcrB was migrated on a 4-15% Tris-glycine SDS-PAGE and the total proteins were Stain-Free™ detected then stained with InstantBlue™ Coomassie. Black arrows indicate the target.

Cryostability

Cryostability was measured using a Tycho (NanoTemper) device that measures changes in intrinsic fluorescence when a thermal ramp is applied. Samples were analyzed after 0 to 3 freeze-thaw cycles (FTC) in liquid nitrogen without addition of cryoprotectant. Two main parameters are measured:

- Sample Brightness: this parameter is linked to the amount of protein present in the sample. If the protein aggregates, a loss in Sample Brightness is observed.
- First derivative ratio: the ratio between 350 nm and 330 nm analyzed here is used to measure inflection points that corresponds to the thermal unfolding of the protein thus giving clues about its correct folding and stability.

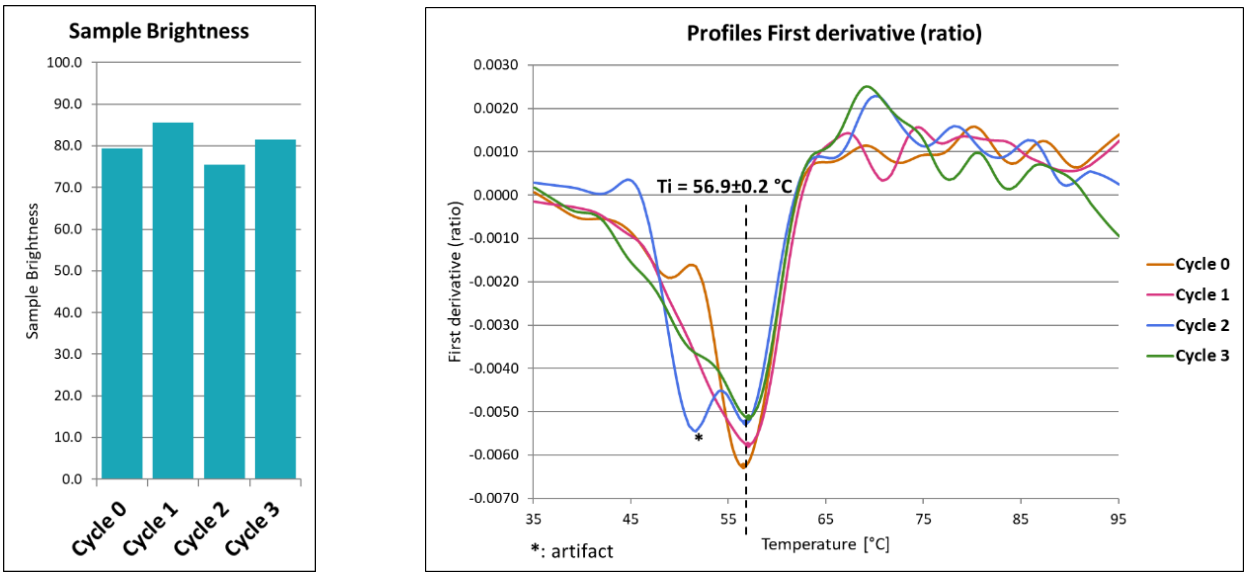
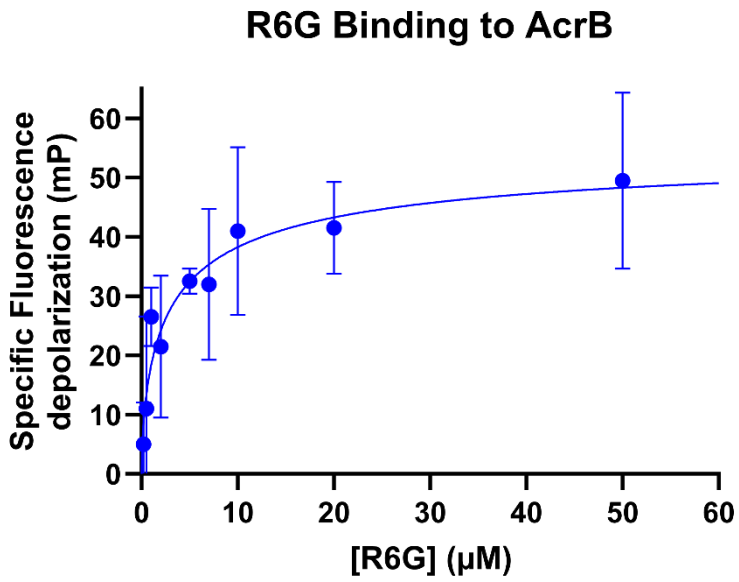


Figure 2: Sample brightness and profiles first derivative profiles measured as the ratio between profiles brightness at 350 nm and 330 nm.

AcrB remains stable in protein buffer after 3 freeze-thaw cycles as shown by Sample Brightness measurements. No inflection temperature was determined as several inflection points are observed on the Profiles First derivative. An inflection temperature of Ti ≃ 57°C was measured that remained stable after 3 freeze-thaw cycles indicating that the protein remains correctly folded.

Ligand Binding Assay

Binding of Rhodamine 6G (R6G) to AcrB was measured by fluorescence polarization assay.



Kd = 3.1 µM

Z' factor = 0.75

Figure 3: Activity measured by Ligand Binding assay.

Recombinant purified AcrB is functional and binds its ligand R6G with an affinity of Kd = 3.1 µM.

References

[1] Eicher T. et al. Transport of drugs by the multidrug transporter AcrB involves an access and a deep binding pocket that are separated by a switch-loop. *Proc Natl Acad Sci U S A*. 2012 Apr 10; 109(15):5687-92.

[2] Pos KM. et al. Purification, crystallization and preliminary diffraction studies of AcrB, an inner-membrane multi-drug efflux protein. *Acta Crystallogr Biol Crystallogr*. 2002; D58 (Pt 10 Pt 2):1865–1867.

Additional Information

Restricted use: Limited Use Label License

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product and progeny, to perform internal research and development for the sole benefit of the purchaser.

Safety

Not known as a hazardous substance or mixture. General industrial hygiene practices must be followed as the use of adapted personal protective equipment for skin and body.

Technical support

For additional product and technical information email our Technical Support team at contact@calixar.com.

Limited product warranty

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