

Materials List for:

High Throughput Quantitative Expression Screening and Purification Applied to Recombinant Disulfide-rich Venom Proteins Produced in *E. coli*

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URL: <https://www.jove.com/video/51464>

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Materials

Name	Company	Catalog Number	Comments
Tecan Freedom EVO 200 liquid handling robot	Tecan Group Ltd.		Protocols can be adapted to any liquid handling robot with a vacuum manifold for plates. http://www.tecan.com/platform/apps/product/index.asp?MenuID=2694&ID=5270&Menu=1&Item=21.1
96-well PCR (PCR96) plates	Greiner Bio-One	652270	http://us.gbo.com/bioscience/detail/2504/
LB medium			Autoclaved for sterility.
Antibiotic stocks			Kanamycin (50 mg/ml), ampicillin (100 mg/ml), chloramphenicol (34 mg/ml in ethanol), store stocks at -20 °C. Use a 1:1,000 dilution.
Deep-well 96 (DW96) plate with 2.42 ml volume capacity	Greiner Bio-One	780270	Autoclaved for sterility. http://us.gbo.com/bioscience/detail/2462/
Expression vectors			For the expression of target constructs. Store at -20 °C.
BL21 (DE3) pLysS competent cells			Or alternative <i>E. coli</i> expression strain of the user's choice. Store at -80 °C.
Breathseal breathable adhesive film	Greiner Bio-One	676050	
PCR machine with 96-well plate block			For the transformation heat shock and boiling of SDS-PAGE/Caliper samples.
24-well sterile tissue culture plates	Greiner Bio-One	662165	http://us.gbo.com/bioscience/detail/2220/
LB-agar			Autoclaved for sterility.
200 µl sterile disposable tips	Tecan Group Ltd.	30 038 617	http://www.tecan.com/platform/apps/product/index.asp?MenuID=2283&ID=4118&Menu=1&Item=21.4
Multitron shaking incubator, with 3 mm throw	Infors	AJ103	Not essential, a regular shaking incubator can also be used. http://www.infors-ht.com/index.php/en/
Plate incubator			
Microtiter plate			For glycerol stocks and elution using purification protocol B.
Glycerol			For the preparation of glycerol stocks.

200 µl disposable tips	Tecan Group Ltd.	30 038 616	http://www.tecan.com/platform/apps/product/index.asp?MenuID=2283&ID=4118&Menu=1&Item=21.4
Adhesive tape pads	Qiagen	19570	http://www.qiagen.com/Products/Catalog/Lab-Essentials-and-Accessories/Tape-Pads
Bactinyl	Orapi Group		Or equivalent microbial disinfectant.
ZYP-5052 medium			See Table 1 for recipes of components.
Deep well 24 (DW24) plates, 10 ml capacity	Whatman	7701-5102	Autoclaved for sterility. http://www.whatman.com/UNIPLATECollectionandAnalysisMicroplates.a
Flat-bottomed, clear microtiter plate	Greiner Bio-One	655101	For absorbance readings. http://us.gbo.com/bioscience/detail/2029/
Plate reading spectrophotometer			Optional. For measuring OD ₆₀₀ of cultures.
Centrifuge with rotor for deep well plates			Suitable for 3,800 x g.
Lysozyme			50 mg/ml in water. Store at -20 °C.
Imidazole ACS grade	Merck	IX0005-1	A high quality grade of imidazole must be used so that it will not interfere with A ₂₈₀ readings for calculating protein yield.
Lysis buffer			50 mM Tris, 300 mM NaCl, 10 mM imidazole pH 8 containing 0.25 mg/ml lysozyme (or your preferred buffer). Add lysozyme from stocks each time and do not store for extended periods.
Water bath			
Plate sonicator (Ultrasonic processor XL, adapted for deep well plates)	Misonix Inc.		Not essential. Lysozyme alone is normally sufficient for nearly complete lysis.
DNase			2 mg/ml stock in water, filter sterilized. Store aliquots at -20 °C.
Magnesium sulphate (MgSO ₄)			2 M stock in water, autoclaved.
SDS-PAGE or Caliper sample buffer			
Binding buffer			50 mM Tris, 300 mM NaCl, 10 mM imidazole pH 8 (or your preferred buffer). Store at 4 °C.
Wash buffer			50 mM Tris, 300 mM NaCl, 50 mM imidazole pH 8 (or your preferred buffer). Store at 4 °C.
Elution buffer			50 mM Tris, 300 mM NaCl, 250 mM imidazole pH 8 (or your preferred buffer). Store at 4 °C.
96-well Receiver/Filter Plate 20 µm, 1.8 ml capacity	Macherey-Nagel	740686.4	http://www.mn-net.com/ProductsBioanalysis/Accessories/tabid/10909/language/en-US/Default.aspx
200 µl disposable wide bore tips	Tecan Group Ltd.	30 050 348	http://www.tecan.com/platform/apps/product/index.asp?MenuID=2283&ID=4118&Menu=1&Item=21.4

Ni Sepharose 6 Fast Flow resin	GE Healthcare	17-5318-02	For purification of target fusion proteins. Store at 4 °C. Wash and equilibrate before use. http://www.gelifesciences.com/webapp/wcs/stores/servlet/productById/en/GELifeSciences/17531802
Tobacco Etch Virus (TEV) protease			Optional, for cleavage. 2 mg/ml, without reducing agents in storage buffer. Store at -80 °C.
96-well 0.22 µm filter plate	Millipore	MSGV N22 10	Optional. To filter the soluble fraction after cleavage. http://www.millipore.com/catalogue/item/msgvn2210
SDS-PAGE/dot blot equipment or Caliper Labchip GXII equipment			Electrophoresis apparatus and choice of gel type is at the user's discretion.
Spectrophotometer and cuvettes			For measuring absorbance at 280 nm (A_{280}) to calculate yield of soluble proteins. Not required if the analysis is done on the Caliper.
ZipTip pipette tips	Millipore		Optional. The type of ZipTip is at the user's discretion. C4 resin should be used for larger proteins, while for peptides C18 may be better. Alternative methods for desalting of samples may also be used to clean up samples before further quality control. http://www.millipore.com/catalogue/module/c5737#0
Materials are listed in the order in which they are required in the Protocol section. Reagents can be stored at room temperature unless noted otherwise. Reference numbers for the author's preferred choice of materials are provided, however equivalent products may also be suitable. For reagents where the brand will not influence the outcome of the experiment, the company details have been omitted.			