

Materials List for:

Production, Crystallization and Structure Determination of *C. difficile* PPEP-1 via Microseeding and Zinc-SAD

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URL: <https://www.jove.com/video/55022>

DOI: [doi:10.3791/55022](https://doi.org/10.3791/55022)

Materials

Name	Company	Catalog Number	Comments
Genes / Vectors / cell strains			
pET28a vector	Merck-Millipore	69864	Thrombin cleavable N-terminal His-tag
<i>E. coli</i> strain BL21 (DE3) Star	ThermoFisher Scientific	C601003	RNase H deficient
Codon-optimized gene (for <i>E. coli</i>) of PPEP-1 (CD630_28300)	Geneart (Thermo Fisher Scientific)	custom	amino acids 27-220
Name	Company	Catalog Number	Comments
Chemicals			
Yeast extract	any		
Tryptone	any		
Antifoam B	Sigma-Aldrich	A5757	aqueous-silicone emulsion
Agar	any		
Kanamycin	any		
IPTG	AppliChem	A1008	
Tris-HCl	AppliChem	A1087	Buffer grade
NaCl	any		Buffer grade
DNaseI	AppliChem	A3778	
Imidazole	AppliChem	A1073	Buffer grade
Thrombin	Sigma-Aldrich	T4648	
Ammonium phosphate dibasic	Sigma-Aldrich	215996	
Glycerol 100%	any		purest grade
Sucrose	Sigma-Aldrich	84097	
Liquid nitrogen	any		for storage and cryocooling of crystals
Name	Company	Catalog Number	Comments
Equipment (general)			
Shaking incubator	any		providing temperatures of 20 °C - 37 °C
Glassware	any		baffled Erlenmeyer flasks (50 ml - 2.8 L)
Centrifuge for large culture volumes	any		centrifuge for processing volumes up to 12 L
Sonicator Vibra-Cell VCX500	Sonics	SO-VCX500	or any other sonicator / cell disruptor
Ultracentrifuge	any		centrifuge providing speeds up to 150,000 x g
NiNTA Superflow resin	Qiagen		

Empty Glass Econo-Column	Bio-Rad	7371007	or any other empty glass or plastic column
Size exclusion chromatography column HiLoad Superdex 200 16/600	GE Healthcare	28989335	
Chromatography system Äkta Purifier	GE Healthcare	28406264	or any other chromatography system
Dialysis tubing Spectra/Por 3	Spectrum Labs	132724	
Dialysis tubing closures	Spectrum Labs	132738	
Ultrafiltration units (concentrators) 10,000 NWCO	any		
UV-Vis spectrophotometer	any		
Name	Company	Catalog Number	Comments
Equipment (crystallography)			
Low volume pipette 0.1-10 µl	any		
Positive displacement pipette Microman M10	Gilson	F148501	
Crystallization robot	any		
96-well crystallization plates TTP IQ with three protein wells	TTP	4150-05810	or any other 96-well crystallization plate
24-well CombiClover Junior Plate	Jena Bioscience	EB-CJR	
Crystal Clear Sealing Tape	Hampton Research	HR3-511	
Siliconized Glass Cover Slides	Hampton Research	HR3-225	
Commercial crystallization screens: SaltRx, Index, PEG/Ion, Crystal	Hampton Research	diverse	
Commercial crystallization screens: Wizard, PACT++, JCSG++	Jena Bioscience	diverse	
JBS Beads-for-Seeds	Jena Bioscience	CO-501	
CrystalCap SPINE HT (nylon loops)	Hampton Research	diverse	loop sizes 0.025 mm - 0.5 mm
CrystalCap Vial	Hampton Research	HR4-904	
Cryogenic Foam Dewar 800 ml	Hampton Research	HR4-673	
Cryogenic Foam Dewar 2 L	Hampton Research	HR4-675	
Vial Clamp, Straight	Hampton Research	HR4-670	
CrystalWand Magnetic, Straight	Hampton Research	HR4-729	
CryoCane 6 Vial Holder	Hampton Research	HR4-711	
CryoSleeve	Hampton Research	HR4-708	
CryoCane Color Coder - White	Hampton Research	HR4-713	
Scalpel	any		
Straight microforcep	any		for manipulation of sealing tape. etc.
Acupuncture needle	any		e.g. from a pharmacy
Stereo microscope	any		for inspection of crystallization plates and crystal mounting, magnification up to 160X