

ProPlex HT-96

MD1-42

ProPlex is formulated for the crystallization of **Protein comPlexes**.

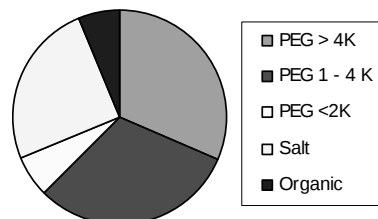
Developed and tested by Sergei Radaev *et al*, at the NIH National Institute of Allergy and Infectious Diseases.
The kit contains a 96 × 1ml deep-well block, targeted sparse matrix screen

Features of ProPlex

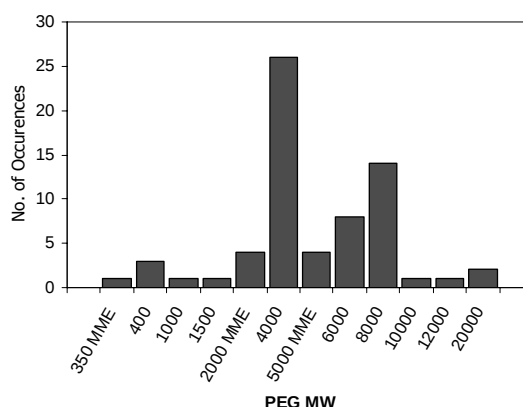
- Targeted sparse matrix screen.
- Based on results of Protein Complex Crystallization Database.
- Satisfies dual requirements:
- Maintain protein-protein interactions,
- Reduce solubility of complex.
- Medium and High MW PEGs.
- Lower PEG concentrations.
- Fewer organic precipitants.
- Neutralised organic acids.
- pH range from 4.0 – 8.5.

Crystallization of protein complexes

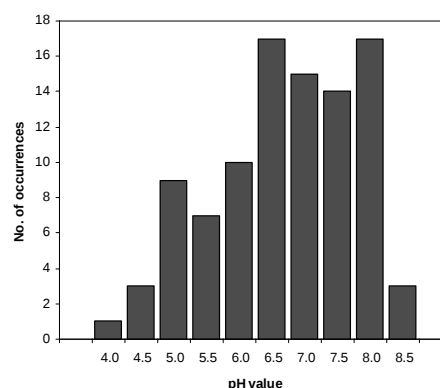
Successful crystallization of protein-protein complexes requires conditions to satisfy two independent criteria – solubility of the complex and stability of the complex. Protein-protein interactions are often weak or transient and their satisfaction precludes a number of reagent zones, thus constricting boundaries around which conditions for crystallization of the complex may be found. Compared to comprehensive crystallization databases containing all proteins, fewer complexes crystallize at extreme pH values due to the destabilisation of protein-protein interactions. Protein complexes were also found to crystallize at lower concentrations of precipitant than is generally observed. As a consequence, traditional sparse matrix screens contain many conditions which fall outside these boundaries and therefore can never crystallize intact protein complexes.



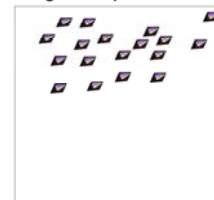
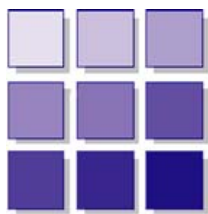
Types of precipitants used for protein-protein complex crystallization



Typical PEG Molecular Weights used in protein-protein complex crystallization.



Typical pH conditions used for protein-protein complex crystallization.



The protein-complex crystallization database

The protein-complex crystallization database (PCCD) was established by Radaev *et al* (2006). All published protein-protein complex structures were extracted from the PDB, and multi-subunit proteins, such as free antibodies, were excluded. The resulting PCCD contained 659 unique, dissociable protein-protein complexes. They included 155 enzyme-inhibitor complexes, 121 receptor-ligand complexes, 117 cellular protein complexes, 74 antibody-antigen complexes, 71 signal transduction complexes, 52 large, multi-protein complexes such as ribosomes, and 69 other types of protein-protein complexes. Analysis of crystallization conditions in the PCCD enabled the definition of crystallization boundaries specific to protein complexes.

The development of *ProPlex*

This *Protein Complex Screen* is a sparse matrix screen containing conditions obtained by cluster-analysis of data from the PCCD. The number of conditions containing each precipitant type is proportional to the number of observed crystallizations in the PCCD: 66 PEG-based, 24 salt-based and 6 organics-based.

Conditions included contain precipitants at concentrations representative of those within the crystallization space identified from the PCCD. These are on average, lower than the concentrations found in general sparse matrix screens.

Screening for crystallization of protein complexes

Analysis of the PCCD revealed that 96% of the crystallizations used the vapour diffusion method. Crystallization experiments should be set-up in parallel at 4 °C and 23 °C, since the strength of interactions at protein-protein interfaces are temperature dependent. Most protein complexes were crystallized at a concentration between 5 and 20 mg/ml, with 10 mg/ml being the most successful starting concentration.

Careful biophysical characterisation of the sample is recommended in order to confirm the nature and stability of the complex.

Formulation Notes

ProPlex reagents are formulated using the highest purity chemicals and ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added.

Buffer stock solutions and organic acids are titrated to the specified pH using either HCl or NaOH. Final pH may vary from that specified on the datasheet. Molecular Dimensions will be happy to discuss the precise formulation of individual reagents.

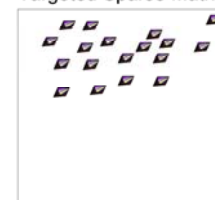
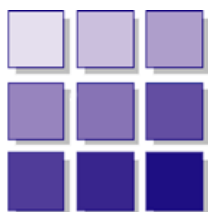
Individual reagents and stock solutions for optimization are available from Molecular Dimensions.

Enquiries regarding ProPlex formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

Contact and product details can be found at www.moleculardimensions.com

References

1. Radaev, S., Li, S. and Sun, P. D. (2006) A survey of protein-protein complex crystallizations. *Acta Cryst.* **D62** pp 605-612
2. Radaev & Sun (2002) Crystallization of Protein-protein complexes. *J. Appl. Cryst.* **35** pp 674-676
3. Dafforn (2007) So how do you know you have a macromolecular complex? *Acta Cryst.* **D63** pp 17-25
4. Crystallization of Nucleic Acids and Proteins, Edited by A. Ducruix and R. Giegé, The Practical Approach Series, Oxford Univ. Press, 1992
5. Protein Crystallization Techniques Strategies & Tips, Edited by Terese Bergfors, IUL 1999.

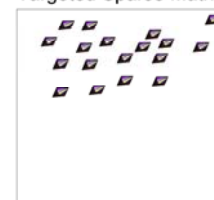
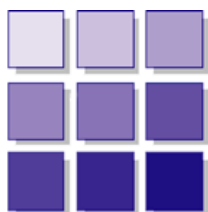


ProPlex Screen HT-96

Rows A - D

MD1-42

HT-96 Well	Salt	Buffer	pH	Precipitant	Additive
A1	None	0.1 M Tris	8.0	25 % v/v PEG 350 MME	None
A2	0.1 M calcium acetate	0.1 M MES	6.0	15 % v/v PEG 400	None
A3	0.1 M lithium Chloride	0.1 M Na HEPES	7.5	20 % v/v PEG 400	None
A4	None	0.1 M Tris	8.0	25 % v/v PEG 400	None
A5	None	0.1 M MES	6.5	15 % v/v PEG 550 MME	None
A6	0.2 M sodium chloride	0.1 M Na/K phosphate	6.5	25 % w/v PEG 1000	None
A7	0.1 M ammonium sulfate	0.1 M Tris	7.5	20 % w/v PEG 1500	None
A8	0.2 M ammonium sulfate	0.1 M sodium acetate	5.5	10 % w/v PEG 2000 MME	None
A9	0.2 M sodium chloride	0.1 M MES	6.0	20 % w/v PEG 2000 MME	None
A10	0.1 M potassium chloride	0.1 M Tris	8.0	15 % w/v PEG 2000 MME	None
A11	None	0.1 M Na HEPES	7.5	25 % w/v PEG 2000 MME	None
A12	0.2 M sodium acetate	0.1 M sodium citrate	5.5	5 % w/v PEG 4000	None
B1	0.2 M lithium sulfate	0.1 M Tris	7.5	5 % w/v PEG 4000	None
B2	0.1 M calcium acetate	0.1 M sodium acetate	4.5	10 % w/v PEG 4000	None
B3	0.2 M sodium acetate	0.1 M sodium citrate	5.5	10 % w/v PEG 4000	None
B4	0.2 M sodium chloride	0.1 M MES	6.5	10 % w/v PEG 4000	None
B5	0.1 M magnesium chloride	0.1 M Na HEPES	7.5	10 % w/v PEG 4000	None
B6	None	0.1 M Na HEPES	7.0	10 % w/v PEG 4000	10 % v/v 2-propanol
B7	0.2 M ammonium acetate	0.1 M sodium acetate	4.0	15 % w/v PEG 4000	None
B8	0.1 M magnesium chloride	0.1 M sodium citrate	5.0	15 % w/v PEG 4000	None
B9	None	0.1 M sodium cacodylate	6.0	15 % w/v PEG 4000	None
B10	0.15 M ammonium sulfate	0.1 M MES	6.0	15 % w/v PEG 4000	None
B11	None	0.1 M Na HEPES	7.0	15 % w/v PEG 4000	None
B12	0.1 M magnesium chloride	0.1 M Na HEPES	7.0	15 % w/v PEG 4000	None
C1	0.15 M ammonium sulfate	0.1 M Tris	8.0	15 % w/v PEG 4000	None
C2	None	0.1 M sodium citrate	4.5	20 % w/v PEG 4000	None
C3	0.2 M ammonium acetate	0.1 M sodium acetate	5.0	20 % w/v PEG 4000	None
C4	0.2 M lithium sulfate	0.1 M MES	6.0	20 % w/v PEG 4000	None
C5	None	0.1 M Tris	8.0	20 % w/v PEG 4000	None
C6	0.15 M ammonium sulfate	0.1 M Na HEPES	7.0	20 % w/v PEG 4000	None
C7	None	0.1 M sodium citrate	5.6	20 % w/v PEG 4000	20 % v/v 2-propanol
C8	0.2 M sodium chloride	0.1 M Tris	8.0	20 % w/v PEG 4000	None
C9	None	0.1 M sodium cacodylate	5.5	25 % w/v PEG 4000	None
C10	0.15 M ammonium sulfate	0.1 M MES	5.5	25 % w/v PEG 4000	None
C11	None	0.1 M sodium cacodylate	6.5	25 % w/v PEG 4000	None
C12	0.2 M potassium iodide	0.1 M MES	6.5	25 % w/v PEG 4000	None
D1	0.2 M sodium chloride	0.1 M Na HEPES	7.5	25 % w/v PEG 4000	None
D2	None	0.1 M MES	6.5	10 % w/v PEG 5000 MME	12 % v/v 1-propanol
D3	0.1 M potassium chloride	0.1 M Na HEPES	7.0	15 % w/v PEG 5000 MME	None
D4	0.2 M ammonium sulfate	0.1 M Tris	7.5	20 % w/v PEG 5000 MME	None
D5	0.1 M magnesium chloride	0.1 M MES	6.0	8 % w/v PEG 6000	None
D6	0.15 M sodium chloride	0.1 M Tris	8.0	8 % w/v PEG 6000	None
D7	None	0.1 M sodium citrate	5.5	15 % w/v PEG 6000	None
D8	0.1 M magnesium acetate	0.1 M sodium cacodylate	6.5	15 % w/v PEG 6000	None
D9	None	0.1 M MES	6.5	15 % w/v PEG 6000	5 % v/v MPD
D10	0.1 M potassium chloride	0.1 M Na HEPES	7.5	15 % w/v PEG 6000	None
D11	None	0.1 M Tris	8.5	15 % w/v PEG 6000	None
D12	None	0.1 M Tris	8.5	20 % w/v PEG 6000	None



ProPlex Screen HT-96

Rows E - H

MD1-42

HT-96 Well	Salt	Buffer	pH	Precipitant	Additive
E1	0.1 M magnesium acetate	0.1 M sodium acetate	4.5	8 % w/v PEG 8000	None
E2	None	0.1 M sodium citrate	5.0	8 % w/v PEG 8000	None
E3	0.2 M sodium chloride	0.1 M sodium cacodylate	6.0	8 % w/v PEG 8000	None
E4	None	0.1 M Na HEPES	7.0	8 % w/v PEG 8000	None
E5	None	0.1 M Tris	8.0	8 % w/v PEG 8000	None
E6	0.1 M calcium acetate	0.1 M sodium cacodylate	5.5	12 % w/v PEG 8000	None
E7	None	0.1 M sodium phosphate	6.5	12 % w/v PEG 8000	None
E8	0.1 M magnesium acetate	0.1 M MOPS	7.5	12 % w/v PEG 8000	None
E9	0.2 M sodium chloride	0.1 M Na HEPES	7.5	12 % w/v PEG 8000	None
E10	0.2 M ammonium sulfate	0.1 M Tris	8.5	12 % w/v PEG 8000	None
E11	None	0.1 M sodium citrate	5.0	20 % w/v PEG 8000	None
E12	0.2 M ammonium sulfate	0.1 M MES	6.5	20 % w/v PEG 8000	None
F1	None	0.1 M Na HEPES	7.0	20 % w/v PEG 8000	None
F2	0.2 M lithium chloride	0.1 M Tris	8.0	20 % w/v PEG 8000	None
F3	0.1 M magnesium acetate	0.1 M MES	6.5	10 % w/v PEG 10 000	None
F4	None	0.1 M Na HEPES	7.0	18 % w/v PEG 12 000	None
F5	0.1 M sodium chloride	0.1 M Tris	8.0	8 % w/v PEG 20 000	None
F6	None	0.1 M Na HEPES	7.0	15 % w/v PEG 20 000	None
F7	None	0.1 M MES	6.5	0.5 M ammonium sulfate	None
F8	None	0.1 M sodium acetate	5.0	1 M ammonium sulfate	None
F9	None	0.1 M MES	6.5	1 M ammonium sulfate	None
F10	None	0.1 M Tris	8.0	1 M ammonium sulfate	None
F11	None	0.1 M sodium acetate	5.0	1.5 M ammonium sulfate	None
F12	None	0.1 M Na HEPES	7.0	1.5 M ammonium sulfate	None
G1	None	0.1 M Tris	8.0	1.5 M ammonium sulfate	None
G2	None	0.1 M sodium acetate	5.0	2 M ammonium sulfate	None
G3	None	0.1 M Na HEPES	7.0	2 M ammonium sulfate	None
G4	None	0.1 M Tris	8.0	2 M ammonium sulfate	None
G5	1 M potassium chloride	0.1 M Na HEPES	7.0	1 M ammonium sulfate	None
G6	None	0.1 M sodium acetate	5.0	2 M sodium formate	None
G7	None	0.1 M Tris	7.5	3 M sodium formate	None
G8	None	None	7.5	0.8 M Na/K hydrogen phosphate	None
G9	None	None	7.0	1.3 M Na/K hydrogen phosphate	None
G10	None	None	6.5	1.6 M Na/K hydrogen phosphate	None
G11	None	0.1 M Na HEPES	7.5	1 M sodium acetate	None
G12	None	0.1 M Na HEPES	7.0	1 M sodium citrate	None
H1	None	0.1 M sodium citrate	6.0	2 M sodium chloride	None
H2	None	0.1 M MES	6.5	1 M lithium sulfate	None
H3	None	0.1 M Tris	8.0	1.6 M lithium sulfate	None
H4	None	None	6.0	1.4 M sodium malonate	None
H5	None	0.1 M Tris	8.0	1.2 M Na/K tartrate	None
H6	None	0.1 M MES	6.5	1.6 M magnesium sulfate	None
H7	None	0.1 M sodium acetate	5.0	15 % v/v MPD	2 % w/v PEG 4000
H8	0.05 M calcium acetate	0.1 M sodium cacodylate	6.0	25 % v/v MPD	None
H9	None	0.1 M imidazole	7.0	50 % v/v MPD	None
H10	0.05 M magnesium Chloride	0.1 M MES	6.5	10 % v/v 2-propanol	5 % w/v PEG 4000
H11	0.2 M ammonium acetate	0.1 M Na HEPES	7.5	25 % v/v 2-propanol	None
H12	0.1 M sodium chloride	0.1 M Tris	8.0	15 % v/v ethanol	5 % v/v MPD

Abbreviations:

HEPES; N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid, MES; 2-(N-morpholino)ethanesulfonic acid, MME; Monomethylether, PEG; Polyethylene glycol, Tris; 2-Amino-2-(hydroxymethyl)propane-1,3-diol, MOPS; 3-(N-Morpholino)-propanesulfonic acid;