

PGA-LM Crystallization Screen™MD1-50

A novel precipitant and a totally new crystallization space to explore.

A revolutionary new systematic screen from the York Structural Biology Laboratory (YSBL) based on the poly- γ -glutamic acid (PGA) polymer. A screen suitable for both globular and membrane protein crystallization.

The kit contains 96 \times 10ml conditions.

Features of the PGA-LM screen:

- A stand-alone new protein precipitant.
- Easy mixing properties with other PEGs.
- Suitable for globular and membrane protein crystallization.
- Non-toxic and non-denaturing.
- Compatible with liquid—handling robots.
- Easy mixing properties with other PEGs.

Introduction

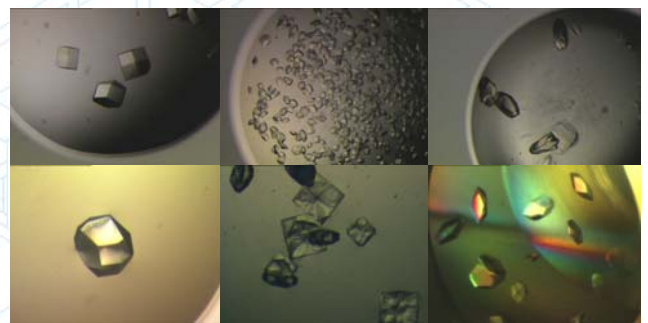
PGA polymers were tested and developed to extend the chemical palette of organic precipitants in macromolecule crystallization. Although the chemical modifications of PGA are not trivial and cost-effective, the original PGAs have been found to be useful in crystallization of globular and membrane proteins without any special treatment.

PGAs present at least two new aspects in protein crystallization; they extend the range of existing PEG-based polymers into (a) new-chemistry type of polymers that exploit poly-amino acids, and (b) widen-up the range of molecular weight of polymer precipitants into regions over 1MDa.

The high nucleation-precipitation potential of PGAs enables their use at very low concentrations and in combination with classical precipitants, scales down the amount of precipitants necessary for crystal appearance and growth. This feature of PGAs makes them especially useful in applications for labile, easily precipitating proteins.

Although they can be employed for all type/classes of proteins, current experience resulting from work in the YSBL suggests that PGA should be especially effective for crystallization of membrane proteins. Therefore, the PGA-based screens are recommended as targeted screens with membrane proteins as the main/primary subjects of their applicability.

The large range of screens currently available are all based on the same set of precipitants; PEGs, MPDs etc. The PGA screen represents a revolutionary new systematic screen based on PGA-LM (200-400kDa low molecular weight polymer).



Examples of lysozyme crystals grown from a variety of PGA-LM conditions.

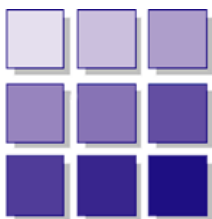


Examples of membrane protein crystals grown from a variety of PGA-LM conditions.

pH control

One of the most important parameters in the crystallization process is pH. The starting pH depends upon prior knowledge of each protein's properties, such as purification characteristics, isoelectric point, solubility/stability, pH-aggregation dependence estimated by dynamic light scattering (DLS) and previous crystallization experience with related proteins.

For example, the sodium cacodylate buffer at pH 6.5 covers a broad plateau of pKa values of individual amino acids and provides additional protection against potential specific protein aggregation caused by free –SH groups.



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The PGA screen (and also the Clear Strategy Screens MD1-14 and MD1-15) have shown that the rational use of pH can accelerate successful crystallogensis through the minimum number of trials.

Instructions for use

PGA screen reagents are formulated using ultrapure water (>18.0 MΩ). No preservatives are added.

- **Final pH may vary from that specified on the datasheet.** As PGA is a polyanionic polymer with chelating properties it is advisable to be mindful of the buffer concentrations of any key ions required by your protein. We **DO NOT** believe it will strip off any metal bound to the protein.

Contact Us

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

For single reagents from PGA use the following catalogue number:

MDSR-50-then insert tube number required here, e.g. MDSR-50-1-24 for tube number 24 of box 1, or MDSR-50-2-24 for tube number 24 of box 2.

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

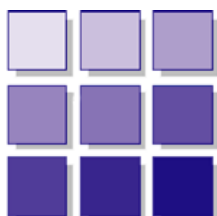
Contact and product details can be found at

moleculardimensions.com

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References

TC Hu, J Korczynska, DK Smith, AM Brzozowski - Acta Crystallographica Section D: Biological Crystallography, 2008. D64, 957-963

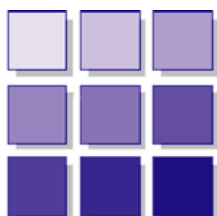


PGA Screen

MD1-50

Box 1 of 2

Tube	Salt 1	Salt 2	Buffer	1 st precipitant	2 nd precipitant	pH
1-1	0.3 M potassium bromide	None	0.1 M sodium acetate	8 % w/v PGA-LM	None	5
1-2	0.2 M magnesium chloride	None	0.1 M sodium acetate	8 % w/v PGA-LM	None	5
1-3	0.3 M sodium malonate	None	0.1 M sodium acetate	8 % w/v PGA-LM	None	5
1-4	0.6 M sodium formate	None	0.1 M sodium acetate	8 % w/v PGA-LM	None	5
1-5	1.0 M ammonium formate	None	0.1 M sodium acetate	8 % w/v PGA-LM	None	5
1-6	0.2 M potassium thiocyanate	None	0.1 M sodium acetate	8 % w/v PGA-LM	None	5
1-7	0.2 M L-proline	None	0.1 M sodium acetate	8 % w/v PGA-LM	None	5
1-8	0.2 M L-arginine	None	0.1 M sodium acetate	8 % w/v PGA-LM	None	5
1-9	None	None	0.1 M sodium acetate	5 % w/v PGA-LM	30 % v/v PEG 400	5
1-10	None	None	0.1 M sodium acetate	5 % w/v PGA-LM	30 % v/v PEG 550MME	5
1-11	None	None	0.1 M sodium acetate	5 % w/v PGA-LM	30 % v/v MPD	5
1-12	None	None	0.1 M sodium acetate	5 % w/v PGA-LM	20 % w/v PEG 2K MME	5
1-13	None	None	0.1 M sodium acetate	5 % w/v PGA-LM	20 % w/v PEG 3350	5
1-14	None	None	0.1 M sodium acetate	5 % w/v PGA-LM	15 % w/v PEG 4K	5
1-15	None	None	0.1 M sodium acetate	5 % w/v PGA-LM	12 % w/v PEG 8K	5
1-16	None	None	0.1 M sodium acetate	5 % w/v PGA-LM	8 % w/v PEG 20K	5
1-17	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium acetate	3 % w/v PGA-LM	30 % v/v PEG 400	5
1-18	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium acetate	3 % w/v PGA-LM	20 % v/v PEG 550 MME	5
1-19	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium acetate	3 % w/v PGA-LM	20 % v/v MPD	5
1-20	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium acetate	3 % w/v PGA-LM	10 % w/v PEG 2K MME	5
1-21	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium acetate	3 % w/v PGA-LM	5 % w/v PEG 3350	5
1-22	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium acetate	3 % w/v PGA-LM	5 % w/v PEG 4K	5
1-23	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium acetate	3 % w/v PGA-LM	5 % w/v PEG 8K	5
1-24	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium acetate	3 % w/v PGA-LM	3 % w/v PEG 20K	5
1-25	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium acetate	3 % w/v PGA-LM	30 % v/v PEG 400	5
1-26	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium acetate	3 % w/v PGA-LM	20 % v/v PEG 550 MME	5
1-27	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium acetate	3 % w/v PGA-LM	20 % v/v MPD	5
1-28	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium acetate	3 % w/v PGA-LM	10 % w/v PEG 2K MME	5
1-29	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium acetate	3 % w/v PGA-LM	5 % w/v PEG 3350	5
1-30	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium acetate	3 % w/v PGA-LM	5 % w/v PEG 4K	5
1-31	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium acetate	3 % w/v PGA-LM	5 % w/v PEG 8K	5
1-32	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium acetate	3 % w/v PGA-LM	3 % w/v PEG 20K	5
1-33	0.3 M potassium bromide	None	0.1 M sodium cacodylate	8 % w/v PGA-LM	None	6.5
1-34	0.2 M magnesium chloride	None	0.1 M sodium cacodylate	8 % w/v PGA-LM	None	6.5
1-35	0.3 M sodium malonate	None	0.1 M sodium cacodylate	8 % w/v PGA-LM	None	6.5
1-36	0.6 M sodium formate	None	0.1 M sodium cacodylate	8 % w/v PGA-LM	None	6.5
1-37	1.0 M ammonium formate	None	0.1 M sodium cacodylate	8 % w/v PGA-LM	None	6.5
1-38	0.2 M potassium thiocyanate	None	0.1 M sodium cacodylate	8 % w/v PGA-LM	None	6.5
1-39	0.2 M L-proline	None	0.1 M sodium cacodylate	8 % w/v PGA-LM	None	6.5
1-40	0.2 M L-arginine	None	0.1 M sodium cacodylate	8 % w/v PGA-LM	None	6.5
1-41	None	None	0.1 M sodium cacodylate	5 % w/v PGA-LM	30 % v/v PEG 400	6.5
1-42	None	None	0.1 M sodium cacodylate	5 % w/v PGA-LM	30 % v/v PEG 550MME	6.5
1-43	None	None	0.1 M sodium cacodylate	5 % w/v PGA-LM	30 % v/v MPD	6.5
1-44	None	None	0.1 M sodium cacodylate	5 % w/v PGA-LM	20 % w/v PEG 2K MME	6.5
1-45	None	None	0.1 M sodium cacodylate	5 % w/v PGA-LM	20 % w/v PEG 3350	6.5
1-46	None	None	0.1 M sodium cacodylate	5 % w/v PGA-LM	15 % w/v PEG 4K	6.5
1-47	None	None	0.1 M sodium cacodylate	5 % w/v PGA-LM	12 % w/v PEG 8K	6.5
1-48	None	None	0.1 M sodium cacodylate	5 % w/v PGA-LM	8 % w/v PEG 20K	6.5



PGA Screen

MD1-50

Box 2 of 2

Tube	Salt 1	Salt 2	Buffer	1 st precipitant	2 nd precipitant	pH
2-1	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium cacodylate	3 % w/v PGA-LM	30 % v/v PEG 400	6.5
2-2	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium cacodylate	3 % w/v PGA-LM	20 % v/v PEG 550 MME	6.5
2-3	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium cacodylate	3 % w/v PGA-LM	20 % v/v MPD	6.5
2-4	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium cacodylate	3 % w/v PGA-LM	10 % w/v PEG 2K MME	6.5
2-5	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium cacodylate	3 % w/v PGA-LM	5 % w/v PEG 3350	6.5
2-6	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium cacodylate	3 % w/v PGA-LM	5 % w/v PEG 4K	6.5
2-7	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium cacodylate	3 % w/v PGA-LM	5 % w/v PEG 8K	6.5
2-8	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M sodium cacodylate	3 % w/v PGA-LM	3 % w/v PEG 20K	6.5
2-9	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium cacodylate	3 % w/v PGA-LM	30 % v/v PEG 400	6.5
2-10	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium cacodylate	3 % w/v PGA-LM	20 % v/v PEG 550 MME	6.5
2-11	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium cacodylate	3 % w/v PGA-LM	20 % v/v MPD	6.5
2-12	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium cacodylate	3 % w/v PGA-LM	10 % w/v PEG 2K MME	6.5
2-13	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium cacodylate	3 % w/v PGA-LM	5 % w/v PEG 3350	6.5
2-14	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium cacodylate	3 % w/v PGA-LM	5 % w/v PEG 4K	6.5
2-15	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium cacodylate	3 % w/v PGA-LM	5 % w/v PEG 8K	6.5
2-16	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M sodium cacodylate	3 % w/v PGA-LM	3 % w/v PEG 20K	6.5
2-17	0.3 M potassium bromide	None	0.1 M Tris	8 % w/v PGA-LM	None	7.8
2-18	0.2 M magnesium chloride	None	0.1 M Tris	8 % w/v PGA-LM	None	7.8
2-19	0.3 M sodium malonate	None	0.1 M Tris	8 % w/v PGA-LM	None	7.8
2-20	0.6 M sodium formate	None	0.1 M Tris	8 % w/v PGA-LM	None	7.8
2-21	1.0 M ammonium formate	None	0.1 M Tris	8 % w/v PGA-LM	None	7.8
2-22	0.2 M potassium thiocyanate	None	0.1 M Tris	8 % w/v PGA-LM	None	7.8
2-23	0.2 M L-proline	None	0.1 M Tris	8 % w/v PGA-LM	None	7.8
2-24	0.2 M L-arginine	None	0.1 M Tris	8 % w/v PGA-LM	None	7.8
2-25	None	None	0.1 M Tris	5 % w/v PGA-LM	30 % v/v PEG 400	7.8
2-26	None	None	0.1 M Tris	5 % w/v PGA-LM	30 % v/v PEG 550MME	7.8
2-27	None	None	0.1 M Tris	5 % w/v PGA-LM	30 % v/v MPD	7.8
2-28	None	None	0.1 M Tris	5 % w/v PGA-LM	20 % w/v PEG 2K MME	7.8
2-29	None	None	0.1 M Tris	5 % w/v PGA-LM	20 % w/v PEG 3350	7.8
2-30	None	None	0.1 M Tris	5 % w/v PGA-LM	15 % w/v PEG 4K	7.8
2-31	None	None	0.1 M Tris	5 % w/v PGA-LM	12 % w/v PEG 8K	7.8
2-32	None	None	0.1 M Tris	5 % w/v PGA-LM	8 % w/v PEG 20K	7.8
2-33	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M Tris	3 % w/v PGA-LM	30 % v/v PEG 400	7.8
2-34	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M Tris	3 % w/v PGA-LM	20 % v/v PEG 550 MME	7.8
2-35	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M Tris	3 % w/v PGA-LM	20 % v/v MPD	7.8
2-36	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M Tris	3 % w/v PGA-LM	10 % w/v PEG 2K MME	7.8
2-37	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M Tris	3 % w/v PGA-LM	5 % w/v PEG 3350	7.8
2-38	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M Tris	3 % w/v PGA-LM	5 % w/v PEG 4K	7.8
2-39	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M Tris	3 % w/v PGA-LM	5 % w/v PEG 8K	7.8
2-40	0.2 M potassium bromide	0.2 M potassium thiocyanate	0.1 M Tris	3 % w/v PGA-LM	3 % w/v PEG 20K	7.8
2-41	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M Tris	3 % w/v PGA-LM	30 % v/v PEG 400	7.8
2-42	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M Tris	3 % w/v PGA-LM	20 % v/v PEG 550 MME	7.8
2-43	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M Tris	3 % w/v PGA-LM	20 % v/v MPD	7.8
2-44	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M Tris	3 % w/v PGA-LM	10 % w/v PEG 2K MME	7.8
2-45	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M Tris	3 % w/v PGA-LM	5 % w/v PEG 3350	7.8
2-46	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M Tris	3 % w/v PGA-LM	5 % w/v PEG 4K	7.8
2-47	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M Tris	3 % w/v PGA-LM	5 % w/v PEG 8K	7.8
2-48	0.1 M ammonium sulfate	0.3 M sodium formate	0.1 M Tris	3 % w/v PGA-LM	3 % w/v PEG 20K	7.8

Abbreviations:

PGA-LM, poly- γ -glutamic acid low molecular weight polymer, **PEG**, polyethylene glycol (concentrations quoted as w/v or v/v%); **MME**, monomethyl ether; 2K, 4K 8K and 20K corresponds to the molecular weight, in thousands of Daltons, of PEG; **MPD**, 2-methyl-2,4-pentanediol;

Manufacturer's safety data sheets are available upon request.