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PACT *premier*™,* MD1-29

PACT *premier* is a pH, Anion, Cation crystallization trial devised to test pH within a PEG/Ion screen environment.

The kit contains 96 reagents arranged as a cation/PEG screen, an anion/PEG screen and a pH/PEG screen

Features of PACT *premier*™

- A modern, comprehensive PEG/ion screen
- This 96 well screen is really 3 screens:
  24-well pH/PEG screen
  24-well cation/PEG screen
  48-well anion/PEG screen

Rationale for a new PEG/ion screen

The first step in crystallization is often to reach for a commercially available "sparse matrix" kit, and hope that one of the conditions produces something that looks harvestable, or optimizable. If no obvious leads come out of the screen, it is hard to learn anything from the negative (precipitate and clear) results.

There are a few screens that try to test crystallization space in a more rational manner – for example, the Clear Strategy Screen and The Solubility Tool Kit.
The PEG/Ion screen (Hampton Research) is a logical test of seven cations and eleven anions using PEG 3350 as the precipitation agent. This screen has been reported by some structural genomics organizations (eg. JCSG, Syrrx) to be very successful in producing crystal hits, with the caveat that many of the hits are redundant – that is, it is not so much the combination of a given cation and anion that is successful, but that either the cation or the anion seems to dominate.

With this in mind, this PEG/ION/pH screen has been developed to systematically test the effect of pH, anions and cations, using PEG as the precipitant. This screen has been implemented very successfully at the Netherlands Cancer Institute (NKI), and at the Oxford Protein Production Facility (OXPPF).

PACT *premier*

pH/PEG screen

This consists of four broad range buffer systems (see Newman, Acta Cryst D, submitted) versus PEG 1500. These buffers allow one to scan the pH range from 4 to 9, without changing the chemistry of the system, so effectively isolating the effect of pH from the effect of the buffer that causes the change in pH.

Cation/PEG screen

This is made up of six cations (all with chloride counter ions) that are combined with PEG 6000 at four different pHs: Acetate pH 5 , MES pH 6 , HEPES pH 7 and Tris pH8 . The cations tested are Na^+, NH_4^+, Li^+, Mg^{2+}, Ca^{2+} and Zn^{2+}. The zinc ion is tested at lower concentration than the other cations in the screen (0.01 M *vs.* 0.2 M)

Anion/PEG screen

This is made up of 12 anions, with either sodium or potassium counter ions, which are tested at 0.2 M against PEG 3350. The anions include fluoride, bromine, iodide, thiocyanate, nitrate, formate, acetate, sulfate, tartrate, phosphate, citrate and malonate. The phosphate solution is tested at a concentration of 0.02 M. Chloride is not included here as it is the counter ion in the cation screen. Three sets of reagents are tested at pH 6.5, 7.5, and 8.5 with the Bis-Tris-Propane buffer system whilst one set of reagents is tested without buffering.

Formulation Notes

PACT *premier* reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added.

Final pH may vary from that specified on the datasheet. Molecular Dimensions will be happy to discuss the precise formulation of individual reagents.

* The screen was developed by Janet Newman, and was tested in the Laboratory of Anastassis Perakis at the NKI as part of the SPINE programme, and is manufactured under license by Molecular Dimensions Ltd.

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

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

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

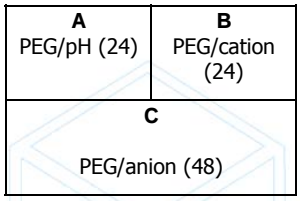
References

1. Newman *et al*, 2005. Towards rationalization of crystallization screening for small- to medium-sized laboratories: the PACT/JCSG+ strategy. Acta Cryst. D61: 1426

To set-up a screen

Take a 96 well plate and divide up into a 24 well pH/PEG screen, a 24 well PEG/cation screen and a 48 well PEG/anion screen as shown in Figure 1. Note: Each subscreen uses a different PEG.

Figure 1



A **PEG / pH screen** 25 % w/v PEG 1500

						SPG system
						MIB system
						PCTP system
						MMT system
4	**5**	**6**	**7**	**8**	**9**	
pH of the buffer system						

B **PEG / cation screen** 20% w/v PEG 6000

						Acetate
						MES
						HEPES
						Tris
NaCl	**NH_4Cl**	**LiCl**	**$MgCl_2$**	**$CaCl_2$**	**$ZnCl_2$**	
0.2M of all, except for 0.01 M $ZnCl_2$.						

C **PEG / anion screen** 20% w/v PEG 3350

												No Buffer
												pH6.5
												pH 7.5
												pH 8.5
NaF	**NaBr**	**NaI**	**KSCN**	**$NaNO_3$**	**Na Formate**	**Na Acetate**	**Na_2SO_4**	**Na / K tartrate**	**Na/KPO_4**	**Na Citrate**	**Na Malonate**	
0.2M of each, except for 0.02 M Na/KPO_4.												0.1M Bis-tris propane

Abbreviations:

SPG system = **S**uccinic Acid, **P**hosphate, **G**lycine system;
MIB system = **M**alonic acid, **I**midazole, **B**oric acid system;
PCTP system = **P**ropionic acid, **C**acodylate, Bis-**t**ris **p**ropane system;
MMT system = **M**alic acid, **M**ES, **T**ris sytem.

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Tube No.	Buffer / Salt	Buffer / Salt	pH	Precipitant
P1-1	0.1 M SPG buffer	None	4.0	25 % w/v PEG 1500
P1-2	0.1 M SPG buffer	None	5.0	25 % w/v PEG 1500
P1-3	0.1 M SPG buffer	None	6.0	25 % w/v PEG 1500
P1-4	0.1 M SPG buffer	None	7.0	25 % w/v PEG 1500
P1-5	0.1 M SPG buffer	None	8.0	25 % w/v PEG 1500
P1-6	0.1 M SPG buffer	None	9.0	25 % w/v PEG 1500
P1-7	0.2 M sodium chloride	0.1 M sodium acetate	5.0	20 % w/v PEG 6000
P1-8	0.2 M ammonium chloride	0.1 M sodium acetate	5.0	20 % w/v PEG 6000
P1-9	0.2 M lithium chloride	0.1 M sodium acetate	5.0	20 % w/v PEG 6000
P1-10	0.2 M magnesium chloride	0.1 M sodium acetate	5.0	20 % w/v PEG 6000
P1-11	0.2 M calcium chloride	0.1 M sodium acetate	5.0	20 % w/v PEG 6000
P1-12	0.01 M zinc chloride	0.1 M sodium acetate	5.0	20 % w/v PEG 6000
P1-13	0.1 M MIB buffer	None	4.0	25 % w/v PEG 1500
P1-14	0.1 M MIB buffer	None	5.0	25 % w/v PEG 1500
P1-15	0.1 M MIB buffer	None	6.0	25 % w/v PEG 1500
P1-16	0.1 M MIB buffer	None	7.0	25 % w/v PEG 1500
P1-17	0.1 M MIB buffer	None	8.0	25 % w/v PEG 1500
P1-18	0.1 M MIB buffer	None	9.0	25 % w/v PEG 1500
P1-19	0.2 M sodium chloride	0.1 M MES	6.0	20 % w/v PEG 6000
P1-20	0.2 M ammonium chloride	0.1 M MES	6.0	20 % w/v PEG 6000
P1-21	0.2 M lithium chloride	0.1 M MES	6.0	20 % w/v PEG 6000
P1-22	0.2 M magnesium chloride	0.1 M MES	6.0	20 % w/v PEG 6000
P1-23	0.2 M calcium chloride	0.1 M MES	6.0	20 % w/v PEG 6000
P1-24	0.01 M zinc chloride	0.1 M MES	6.0	20 % w/v PEG 6000
P1-25	0.1 M PCTP buffer	None	4.0	25 % w/v PEG 1500
P1-26	0.1 M PCTP buffer	None	5.0	25 % w/v PEG 1500
P1-27	0.1 M PCTP buffer	None	6.0	25 % w/v PEG 1500
P1-28	0.1 M PCTP buffer	None	7.0	25 % w/v PEG 1500
P1-29	0.1 M PCTP buffer	None	8.0	25 % w/v PEG 1500
P1-30	0.1 M PCTP buffer	None	9.0	25 % w/v PEG 1500
P1-31	0.2 M sodium chloride	0.1 M HEPES	7.0	20 % w/v PEG 6000
P1-32	0.2 M ammonium chloride	0.1 M HEPES	7.0	20 % w/v PEG 6000
P1-33	0.2 M lithium chloride	0.1 M HEPES	7.0	20 % w/v PEG 6000
P1-34	0.2 M magnesium chloride	0.1 M HEPES	7.0	20 % w/v PEG 6000
P1-35	0.2 M calcium chloride	0.1 M HEPES	7.0	20 % w/v PEG 6000
P1-36	0.01 M zinc chloride	0.1 M HEPES	7.0	20 % w/v PEG 6000
P1-37	0.1 M MMT buffer	None	4.0	25 % w/v PEG 1500
P1-38	0.1 M MMT buffer	None	5.0	25 % w/v PEG 1500
P1-39	0.1 M MMT buffer	None	6.0	25 % w/v PEG 1500
P1-40	0.1 M MMT buffer	None	7.0	25 % w/v PEG 1500
P1-41	0.1 M MMT buffer	None	8.0	25 % w/v PEG 1500
P1-42	0.1 M MMT buffer	None	9.0	25 % w/v PEG 1500
P1-43	0.2 M sodium chloride	0.1 M Tris	8.0	20 % w/v PEG 6000
P1-44	0.2 M ammonium chloride	0.1 M Tris	8.0	20 % w/v PEG 6000
P1-45	0.2 M lithium chloride	0.1 M Tris	8.0	20 % w/v PEG 6000
P1-46	0.2 M magnesium chloride	0.1 M Tris	8.0	20 % w/v PEG 6000
P1-47	0.2 M calcium chloride	0.1 M Tris	8.0	20 % w/v PEG 6000
P1-48	0.002 M zinc chloride	0.1 M Tris	8.0	20 % w/v PEG 6000

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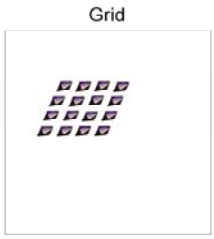
Tube No.	Salt	Buffer System	pH	Precipitant
P2-1	0.2 M sodium fluoride	None		20 % w/v PEG 3350
P2-2	0.2 M sodium bromide	None		20 % w/v PEG 3350
P2-3	0.2 M sodium iodide	None		20 % w/v PEG 3350
P2-4	0.2 M potassium thiocyanate	None		20 % w/v PEG 3350
P2-5	0.2 M sodium nitrate	None		20 % w/v PEG 3350
P2-6	0.2 M sodium formate	None		20 % w/v PEG 3350
P2-7	0.2 M sodium acetate	None		20 % w/v PEG 3350
P2-8	0.2 M sodium sulfate	None		20 % w/v PEG 3350
P2-9	0.2 M potassium/sodium tartrate	None		20 % w/v PEG 3350
P2-10	0.02 M sodium/potassium phosphate	None		20 % w/v PEG 3350
P2-11	0.2 M sodium citrate	None		20 % w/v PEG 3350
P2-12	0.2 M sodium malonate	None		20 % w/v PEG 3350
P2-13	0.2 M sodium fluoride	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
P2-14	0.2 M sodium bromide	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
P2-15	0.2 M sodium iodide	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
P2-16	0.2 M potassium thiocyanate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
P2-17	0.2 M sodium nitrate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
P2-18	0.2 M sodium formate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
P2-19	0.2 M sodium acetate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
P2-20	0.2 M sodium sulfate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
P2-21	0.2 M potassium/sodium tartrate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
P2-22	0.02 M sodium/potassium phosphate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
P2-23	0.2 M sodium citrate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
P2-24	0.2 M sodium malonate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
P2-25	0.2 M sodium fluoride	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
P2-26	0.2 M sodium bromide	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
P2-27	0.2 M sodium iodide	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
P2-28	0.2 M potassium thiocyanate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
P2-29	0.2 M sodium nitrate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
P2-30	0.2 M sodium formate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
P2-31	0.2 M sodium acetate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
P2-32	0.2 M sodium sulfate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
P2-33	0.2 M potassium/sodium tartrate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
P2-34	0.02 M sodium/potassium phosphate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
P2-35	0.2 M sodium citrate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
P2-36	0.2 M sodium malonate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
P2-37	0.2 M sodium fluoride	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
P2-38	0.2 M sodium bromide	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
P2-39	0.2 M sodium iodide	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
P2-40	0.2 M potassium thiocyanate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
P2-41	0.2 M sodium nitrate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
P2-42	0.2 M sodium formate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
P2-43	0.2 M sodium acetate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
P2-44	0.2 M sodium sulfate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
P2-45	0.2 M potassium/sodium tartrate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
P2-46	0.02 M sodium/potassium phosphate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
P2-47	0.2 M sodium citrate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
P2-48	0.2 M sodium malonate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350

Abbreviations:
HEPES; N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid, **MES**; 2-(N-morpholino)ethanesulfonic acid, **PEG**; Polyethylene glycol, **Tris**; 2-Amino-2-(hydroxymethyl)propane-1,3-diol, **SPG** buffer; Succinic Acid, Phosphate, Glycine, **MIB** buffer; Malonic acid, Imidazole, Boric acid, **PCTP** buffer; Propionic acid, Cacodylate, Bis-tris propane, **MMT** buffer; Malic acid, MES, Tris.

Manufacturer's safety data sheets are available upon request.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