

PACT *premier*^{TM,*} HT-96

MD1-36

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

The HT kit contains 96 × 1ml reagents in a deep-well block arranged as a cation/PEG screen, an anion/PEG screen and a pH/PEG screen.

Features of PACT *premier*TM

- 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⁺, NH₄⁺, Li⁺, Mg²⁺, Ca²⁺ and Zn²⁺. 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

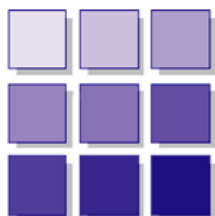
This is made up of 12 anions, with either sodium or potassium counter ions, which are tested at 0.2 M against PEG 3350. The anions include fluoride, bromine, iodide, thiocyanate, nitrate, formate, acetate, sulfate, tartrate, phosphate, citrate and malonate. The phosphate solution is tested at a concentration 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.



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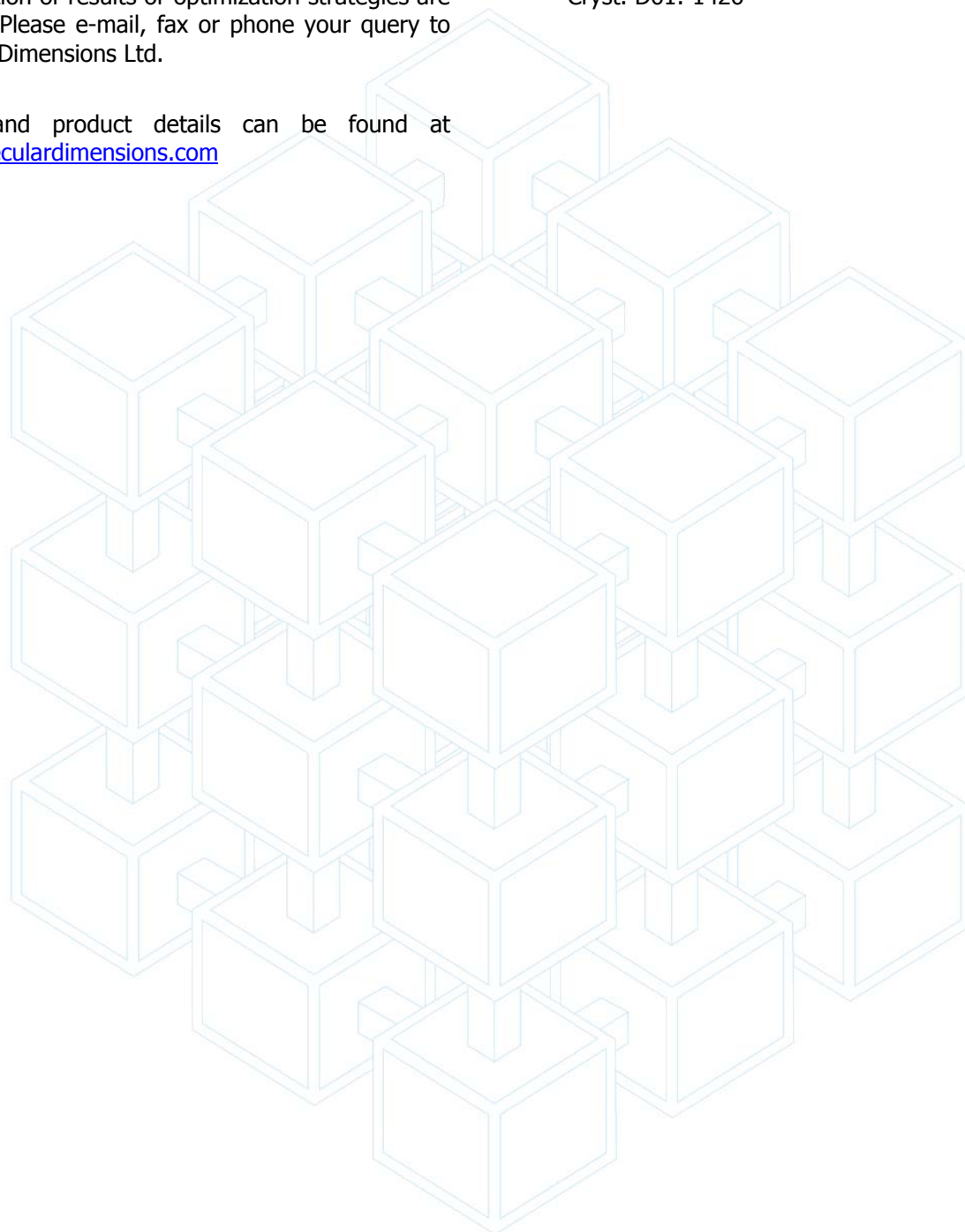
Individual reagents and stock solutions for optimization are available from Molecular Dimensions.

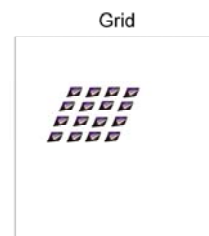
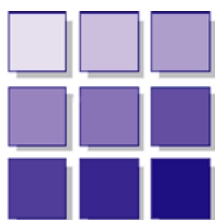
Enquiries regarding PACT *premier* formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions Ltd.

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

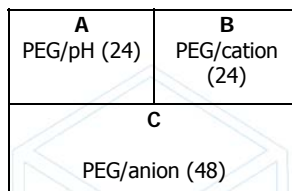




To set-up a screen

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

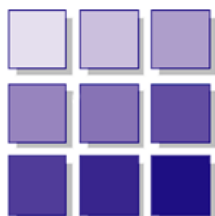
						Acetate
						MES
						HEPES
						Tris
NaCl	NH ₄ Cl	LiCl	MgCl ₂	CaCl ₂	ZnCl ₂	
0.2M of all, except for 0.01 M ZnCl ₂ .						

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

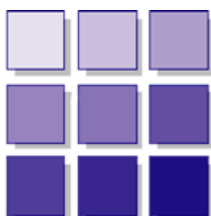
													No Buffer	
													pH 6.5	0.1M Bis-tris propane
													pH 7.5	
													pH 8.5	
NaF	NaBr	NaI	KSCN	NaNO ₃	Na Formate	Na Acetate	Na ₂ SO ₄	Na/K tartrate	Na/KPO ₄	Na Citrate	Na Malonate			
0.2M of each, except for 0.02 M Na/KPO ₄ .														

Abbreviations:

- SPG system = Succinic Acid, Phosphate, Glycine system;
- MIB system = Malonic acid, Imidazole, Boric acid system;
- PCTP system = Propionic acid, Cacodylate, Bis-tris propane system;
- MMT system = Malic acid, MES, Tris system.

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Tube No.	Buffer/Salt	Buffer/Salt	pH	Precipitant
A1	0.1 M SPG buffer	None	4.0	25 % w/v PEG 1500
A2	0.1 M SPG buffer	None	5.0	25 % w/v PEG 1500
A3	0.1 M SPG buffer	None	6.0	25 % w/v PEG 1500
A4	0.1 M SPG buffer	None	7.0	25 % w/v PEG 1500
A5	0.1 M SPG buffer	None	8.0	25 % w/v PEG 1500
A6	0.1 M SPG buffer	None	9.0	25 % w/v PEG 1500
A7	0.2 M sodium chloride	0.1 M sodium acetate	5.0	20 % w/v PEG 6000
A8	0.2 M ammonium chloride	0.1 M sodium acetate	5.0	20 % w/v PEG 6000
A9	0.2 M lithium chloride	0.1 M sodium acetate	5.0	20 % w/v PEG 6000
A10	0.2 M magnesium chloride	0.1 M sodium acetate	5.0	20 % w/v PEG 6000
A11	0.2 M calcium chloride	0.1 M sodium acetate	5.0	20 % w/v PEG 6000
A12	0.01 M zinc chloride	0.1 M sodium acetate	5.0	20 % w/v PEG 6000
B1	0.1 M MIB buffer	None	4.0	25 % w/v PEG 1500
B2	0.1 M MIB buffer	None	5.0	25 % w/v PEG 1500
B3	0.1 M MIB buffer	None	6.0	25 % w/v PEG 1500
B4	0.1 M MIB buffer	None	7.0	25 % w/v PEG 1500
B5	0.1 M MIB buffer	None	8.0	25 % w/v PEG 1500
B6	0.1 M MIB buffer	None	9.0	25 % w/v PEG 1500
B7	0.2 M sodium chloride	0.1 M MES	6.0	20 % w/v PEG 6000
B8	0.2 M ammonium chloride	0.1 M MES	6.0	20 % w/v PEG 6000
B9	0.2 M lithium chloride	0.1 M MES	6.0	20 % w/v PEG 6000
B10	0.2 M magnesium chloride	0.1 M MES	6.0	20 % w/v PEG 6000
B11	0.2 M calcium chloride	0.1 M MES	6.0	20 % w/v PEG 6000
B12	0.01 M zinc chloride	0.1 M MES	6.0	20 % w/v PEG 6000
C1	0.1 M PCTP buffer	None	4.0	25 % w/v PEG 1500
C2	0.1 M PCTP buffer	None	5.0	25 % w/v PEG 1500
C3	0.1 M PCTP buffer	None	6.0	25 % w/v PEG 1500
C4	0.1 M PCTP buffer	None	7.0	25 % w/v PEG 1500
C5	0.1 M PCTP buffer	None	8.0	25 % w/v PEG 1500
C6	0.1 M PCTP buffer	None	9.0	25 % w/v PEG 1500
C7	0.2 M sodium chloride	0.1 M HEPES	7.0	20 % w/v PEG 6000
C8	0.2 M ammonium chloride	0.1 M HEPES	7.0	20 % w/v PEG 6000
C9	0.2 M lithium chloride	0.1 M HEPES	7.0	20 % w/v PEG 6000
C10	0.2 M magnesium chloride	0.1 M HEPES	7.0	20 % w/v PEG 6000
C11	0.2 M calcium chloride	0.1 M HEPES	7.0	20 % w/v PEG 6000
C12	0.01 M zinc chloride	0.1 M HEPES	7.0	20 % w/v PEG 6000
D1	0.1 M MMT buffer	None	4.0	25 % w/v PEG 1500
D2	0.1 M MMT buffer	None	5.0	25 % w/v PEG 1500
D3	0.1 M MMT buffer	None	6.0	25 % w/v PEG 1500
D4	0.1 M MMT buffer	None	7.0	25 % w/v PEG 1500
D5	0.1 M MMT buffer	None	8.0	25 % w/v PEG 1500
D6	0.1 M MMT buffer	None	9.0	25 % w/v PEG 1500
D7	0.2 M sodium chloride	0.1 M Tris	8.0	20 % w/v PEG 6000
D8	0.2 M ammonium chloride	0.1 M Tris	8.0	20 % w/v PEG 6000
D9	0.2 M lithium chloride	0.1 M Tris	8.0	20 % w/v PEG 6000
D10	0.2 M magnesium chloride	0.1 M Tris	8.0	20 % w/v PEG 6000
D11	0.2 M calcium chloride	0.1 M Tris	8.0	20 % w/v PEG 6000
D12	0.002 M zinc chloride	0.1 M Tris	8.0	20 % w/v PEG 6000



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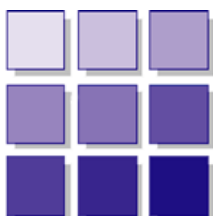
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Tube No.	Salt	Buffer System	pH	Precipitant
E1	0.2 M sodium fluoride	None		20 % w/v PEG 3350
E2	0.2 M sodium bromide	None		20 % w/v PEG 3350
E3	0.2 M sodium iodide	None		20 % w/v PEG 3350
E4	0.2 M potassium thiocyanate	None		20 % w/v PEG 3350
E5	0.2 M sodium nitrate	None		20 % w/v PEG 3350
E6	0.2 M sodium formate	None		20 % w/v PEG 3350
E7	0.2 M sodium acetate	None		20 % w/v PEG 3350
E8	0.2 M sodium sulfate	None		20 % w/v PEG 3350
E9	0.2 M potassium/sodium tartrate	None		20 % w/v PEG 3350
E10	0.02 M sodium/potassium phosphate	None		20 % w/v PEG 3350
E11	0.2 M sodium citrate	None		20 % w/v PEG 3350
E12	0.2 M sodium malonate	None		20 % w/v PEG 3350
F1	0.2 M sodium fluoride	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
F2	0.2 M sodium bromide	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
F3	0.2 M sodium iodide	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
F4	0.2 M potassium thiocyanate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
F5	0.2 M sodium nitrate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
F6	0.2 M sodium formate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
F7	0.2 M sodium acetate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
F8	0.2 M sodium sulfate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
F9	0.2 M potassium/sodium tartrate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
F10	0.02 M sodium/potassium phosphate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
F11	0.2 M sodium citrate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
F12	0.2 M sodium malonate	0.1 M Bis Tris propane	6.5	20 % w/v PEG 3350
G1	0.2 M sodium fluoride	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
G2	0.2 M sodium bromide	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
G3	0.2 M sodium iodide	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
G4	0.2 M potassium thiocyanate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
G5	0.2 M sodium nitrate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
G6	0.2 M sodium formate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
G7	0.2 M sodium acetate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
G8	0.2 M sodium sulfate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
G9	0.2 M potassium/sodium tartrate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
G10	0.02 M sodium/potassium phosphate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
G11	0.2 M sodium citrate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
G12	0.2 M sodium malonate	0.1 M Bis Tris propane	7.5	20 % w/v PEG 3350
H1	0.2 M sodium fluoride	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
H2	0.2 M sodium bromide	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
H3	0.2 M sodium iodide	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
H4	0.2 M potassium thiocyanate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
H5	0.2 M sodium nitrate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
H6	0.2 M sodium formate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
H7	0.2 M sodium acetate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
H8	0.2 M sodium sulfate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
H9	0.2 M potassium/sodium tartrate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
H10	0.02 M sodium/potassium phosphate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
H11	0.2 M sodium citrate	0.1 M Bis Tris propane	8.5	20 % w/v PEG 3350
H12	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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