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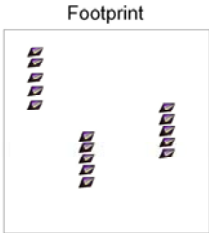


Stura Footprint Screens MD1-20

The footprint screens are based on the concept of screening the protein precipitant solubility curve.

The screens are presented as two sets of 24 conditions (10 ml) at acidic, neutral and basic pH's for polyethylene glycols and salts.

Features of Footprint screening

- Screens the protein precipitant solubility curve rather than a crystallization trial.
- Test the relative protein solubility with precipitants that have been used successfully in the crystallization of many proteins.
- Once initial crystals or crystalline aggregates are obtained from the initial screening, streak seeding is recommended to determine the ranges of conditions under which crystal growth can proceed.

Introduction

A simple screening method to analyse the crystallization potential of proteins and their complexes has been described by Enrico Stura [1]. This method is described as the "First Footprint" as it defines the individual pattern of solubility for a given protein with a range of precipitants and enables the researcher to characterize the types of oils or precipitates obtained. The test is fast and easy to perform as a vapour diffusion experiment. It is highly accurate and reproducible; provided the normal precautions are taken to control the ambient temperature of the experiment. The First Footprint screen is limited to three pH values (5.5, 7.0, and 8.50) and two precipitant classes (PEGs and salts) at four concentrations.

A PEG Footprint screen to compliment the original Footprint Screen has subsequently been described[2]. This Footprint screen covers six pH values (4.5 – 8.2) with six PEG's at four concentrations.

In cases where the amount of protein is limited a systematic approach based on an *a priori* choice of precipitant may be preferable to an extensive search with sparse matrices or factorial approaches. The Reverse Screening Technique[3] can be applied in such situations. The solutions provided in the Footprint screens may be utilised to assist in making that *a priori* choice of precipitant.

A rapid method for using the Footprint solubility screen (with limited amounts of protein).

On the first day set up drop 2C of Footprint Screen #1: dispense the protein (1μl) onto the coverslip/microbridge add the precipitant (1μl). Do not mix. Go immediately to the microscope. As the precipitant and the protein mix together the protein will experience locally the final precipitant concentration. You should see some precipitation within 5 mins. If you see too much precipitate set up 2B and repeat. If after 10 minutes there is absolutely no precipitate, consider setting up 2D or concentrating your protein more. Given that you see some precipitate but not an excessive amount continue to set up 1C, 3C-5C, and 6B of Footprint #1 and/or row 1A, 2B, 3A, 4B, 5A, 6B, of the PEG screen. On the second day set up higher or lower concentrations depending on the results.

Further drops may be set up with PEG at different pH levels to evaluate the change in solubility with pH.

While solubility screening with PEG is normally instantaneous, salts will require equilibration for at least 8 hours.

Note: When perfecting your crystallization conditions it is more accurate to mix 30% of 2B with 70% of 2C rather than mixing a new solution at the intermediate concentration.

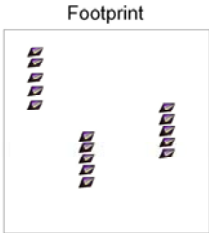
Formulation Notes

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

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 Footprint Screen formulation, interpretation of results or optimization strategies

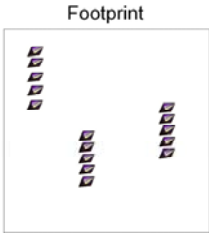


are welcome. Please e-mail, fax or phone your query to Molecular dimensions.

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

References

1. Stura E.A., Nemerow G.R., Wilson I.A (1992) Strategies in the crystallization of glycoproteiens and protein complexes. Journal of Crystal Growth 122:273-285.
2. Stura E.A. (1999) Strategy 3: Reverse Screening. In "Crystallization of Proteins: Techniques, Strategies and Tips. A laboratory manual" (Bergfors T. ed.) International University Line pp113-124.
3. Stura E.A, Satterthwait, A.C, Calvo, J.C, Kaslow, D.C, Wilson, I.A. (1994) Reverse Screening. Acta Cryst. D50 : 448-455.



Stura Footprint Screen #1

	1	2	3	4	5	6
	PEG 600	PEG 4K	PEG 10K	NH_4SO_4	PO_4	Citrate
A	15%	10%	7.5%	0.75M	0.8M	0.75M
B	24%	15%	12.5%	1.0M	1.32M	1.0M
C	33%	20%	17.5%	1.5M	1.6M	1.2M
D	42%	25%	22.5%	2.0M	2.0M	1.5M
	0.2 M Imidazole malate pH 5.5	0.2 M Imidazole malate pH 7.0	0.2 M Imidazole malate pH 8.5	0.15 M Sodium citrate pH 5.5	NaH_2PO_4 K_2HPO_4 pH 7.0	10 mM Sodium borate pH 8.5

Stura PEG Footprint Screen

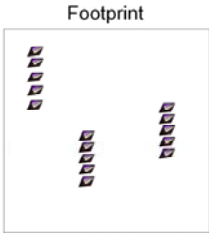
This screen can be set up in full if protein supply is not at a premium. Otherwise proteins generally respond well to 1A, 2B, 3A, 4B, 5A, 6B. Column 4 allows a direct comparison to be made to results obtained with Footprint screen #1 above. The buffers in the other columns have been chosen to be compatible with the use of divalent metals.

Stura PEG Screen Footprint #2

	1	2	3	4	5	6
	PEG 550 MME	PEG 600	PEG 2K MME	PEG 4K	PEG 5K MME	PEG 10K
A	30%	18%	18%	8%	12%	9%
B	40%	27%	27%	15%	18%	15%
C	50%	36%	36%	20%	24%	22.5%
D	60%	45%	45%	30%	36%	27%
	0.1 M HEPES pH 8.2	0.1 M HEPES pH 7.5	0.1 M Sodium cacodylate pH 6.5	0.2 M Imidazole malate pH 6.0	0.1 M Sodium acetate pH 5.5	0.1 M Ammonium acetate pH 4.5

Abbreviations:

HEPES; N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid, **MME**; Monomethylether, **PEG**; Polyethylene glycol.



Stura Footprint Screen MD1-20

Footprint Screen #1					
Tube	**Set**	**Salt/Buffer**	**pH**	**Precipitant**	**Plate Position**
1	1	0.2 M imidazole malate	5.5	15% v/v PEG 600	1A
2	1	0.2 M imidazole malate	5.5	24 %v/v PEG 600	1B
3	1	0.2 M imidazole malate	5.5	33 % v/v PEG 600	1C
4	1	0.2 M imidazole malate	5.5	42 % v/v PEG 600	1D
5	1	0.2 M imidazole malate	7.0	10 % w/v PEG 4000	2A
6	1	0.2 M imidazole malate	7.0	15 % w/v PEG 4000	2B
7	1	0.2 M imidazole malate	7.0	20 % w/v PEG 4000	2C
8	1	0.2 M imidazole malate	7.0	25 % PEG w/v 4000	2D
9	1	0.2 M imidazole malate	8.5	7.5 % w/v PEG 10,000	3A
10	1	0.2 M imidazole malate	8.5	12.5 % w/v PEG 10,000	3B
11	1	0.2 M imidazole malate	8.5	17.5 % w/v PEG 10,000	3C
12	1	0.2 M imidazole malate	8.5	22.5 % w/v PEG 10,000	3D
13	1	0.15 M sodium citrate	5.5	0.75 M ammonium sulfate	4A
14	1	0.15 M sodium citrate	5.5	1.0 M ammonium sulfate	4B
15	1	0.15 M sodium citrate	5.5	1.5 M ammonium sulfate	4C
16	1	0.15 M sodium citrate	5.5	2.0 M ammonium sulfate	4D
17	1	0.8 M NaH_2PO_4/K_2HPO_4	7.0	-	5A
18	1	1.32 M NaH_2PO_4/K_2HPO_4	7.0	-	5B
19	1	1.6 M NaH_2PO_4/K_2HPO_4	7.0	-	5C
20	1	2.0 M NaH_2PO_4/K_2HPO_4	7.0	-	5D
21	1	0.01 M sodium borate	8.5	0.75 M sodium citrate	6A
22	1	0.01 M sodium borate	8.5	1.0 M sodium citrate	6B
23	1	0.01 M sodium borate	8.5	1.2 M sodium citrate	6C
24	1	0.01 M sodium borate	8.5	1.5 M sodium citrate	6D
Footprint Screen #2					
1	2	0.1 M Na HEPES	8.2	30 % v/v PEG 550 MME	1A
2	2	0.1 M Na HEPES	8.2	40 % v/v PEG 550 MME	1B
3	2	0.1 M Na HEPES	8.2	50 % v/v PEG 550 MME	1C
4	2	0.1 M Na HEPES	8.2	60 % v/v PEG 550 MME	1D
5	2	0.1 M Na HEPES	7.5	18 % v/v PEG 600	2A
6	2	0.1 M Na HEPES	7.5	27 % v/v PEG 600	2B
7	2	0.1 M Na HEPES	7.5	36 % v/v PEG 600	2C
8	2	0.1 M Na HEPES	7.5	45 % v/v PEG 600	2D
9	2	0.1 M sodium cacodylate	6.5	18 % w/v PEG 2000 MME	3A
10	2	0.1 M sodium cacodylate	6.5	27 % w/v PEG 2000 MME	3B
11	2	0.1 M sodium cacodylate	6.5	36 % w/v PEG 2000 MME	3C
12	2	0.1 M sodium cacodylate	6.5	45 % w/v PEG 2000 MME	3D
13	2	0.2 M imidazole malate	6.0	8 % w/v PEG 4000	4A
14	2	0.2 M imidazole malate	6.0	15 % w/v PEG 4000	4B
15	2	0.2 M imidazole malate	6.0	20 % w/v PEG 4000	4C
16	2	0.2 M imidazole malate	6.0	30 % w/v PEG 4000	4D
17	2	0.1 M sodium acetate	5.5	12% PEG w/v 5000 MME	5A
18	2	0.1 M sodium acetate	5.5	18% PEG w/v 5000 MME	5B
19	2	0.1 M sodium acetate	5.5	24% PEG w/v 5000 MME	5C
20	2	0.1 M sodium acetate	5.5	36% PEG w/v 5000 MME	5D
21	2	0.1 M ammonium acetate	4.5	9 % w/v PEG 10,000	6A
22	2	0.1 M ammonium acetate	4.5	15 % w/v PEG 10,000	6B
23	2	0.1 M ammonium acetate	4.5	22.5 % w/v PEG 10,000	6C
24	2	0.1 M ammonium acetate	4.5	27 % w/v PEG 10,000	6D

Manufacturer's safety data sheets are available upon request

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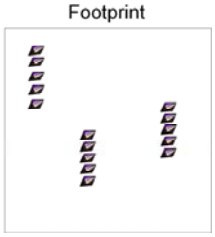


MacroSol™ MD1-22

A Footprint screen for the crystallization of protein complexes and other samples with heterogenous solubility. Screens the protein precipitant solubility curve rather than setting up a randomized crystallization trial.

The screens are presented as two sets of 24 conditions (10 ml) at acidic, neutral and basic pH's for polyethylene glycols and salts.

Features of Footprint screening

- Screens the protein precipitant solubility curve rather than a crystallization trial.
- Test the relative protein solubility with precipitants that have been used successfully in the crystallization of many proteins.
- Once initial crystals or crystalline aggregates are obtained from the initial screening, streak seeding is recommended to determine the ranges of conditions under which crystal growth can proceed.

Introduction

Footprint screens are based on the concept of screening the protein precipitant solubility curve. When crystallizing complexes, each of the macromolecules that compose the complex will have a different solubility. It is therefore important to find out where each of the macromolecules precipitates as well as determining the precipitation points for the complex. Screening under multiple conditions is important in these cases, in particular when the exact stoichiometry for the complex is unknown or the complex has been mixed from the individual solutions rather than purified as a complex. Depending on how much excess of one particular macromolecule is present in the solution we will find some precipitation under the condition for that uncomplexed macromolecule. Hence screening under multiple conditions is needed for complexes, but also for glycoproteins and proteins obtained from limited proteolysis where heterogeneity in the protein will be reflected in solubility differences.

The footprint principle can be applied to any of the commercial screens by making different dilutions of the pre-mixed solutions, thus extending their use and increasing the amount of information than can be derived for each precipitant/buffer mixture without having to mix new solutions. By diluting solutions instead of mixing new ones from scratch it is possible to achieve greater precision. This is the concept of working solutions (2).

This footprint type screen is an example of how one can extend commercial screens and thus make better use of them. It has been taken from the presentation by Dr E. Stura at the "EMBO Workshop on the crystallization of Macromolecular Complexes" Grenoble 8-13 April 2001.

A simple screening method to analyse the crystallization potential of proteins and their complexes has been described by Enrico Stura (1). This method is described as a "Footprint" as it defines the individual pattern of solubility for a given protein with a range of precipitants and enables the researcher to characterize the types of oils or precipitates obtained. The test is fast and easy to perform as a vapour diffusion experiment. It is highly accurate and reproducible; provided the normal precautions are taken to control the ambient temperature of the experiment. MacroSol is a footprint-type screen based on reagents of proven success in crystallizing glycoproteins and macromolecular complexes.

Formulation Notes

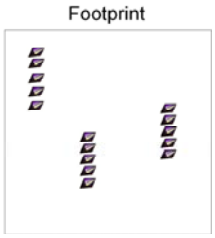
MacroSol reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added.

Final pH may vary from that specified on the datasheet.

Molecular Dimensions will be happy to discuss the precise formulation of individual reagents.

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

Once initial crystals or crystalline aggregates are obtained from the first screening, streak seeding is recommended to determine the ranges of conditions under which crystal growth can proceed.

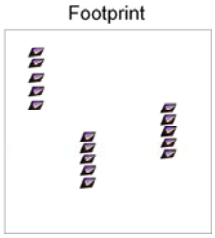


Enquiries regarding MacroSol formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

Contact and product details can be found at moleculardimensions.com

References

1. Stura E.A., Nemerow G.R., Wilson I.A (1992) Strategies in the crystallization of glycoproteiens and protein complexes. Journal of Crystal Growth 122:273-285
2. Stura E.A. (1999) Strategy 3: Reverse Screening. In "Crystallization of Proteins: Techniques, Strategies and Tips. A laboratory manual" (Bergfors T. ed.) International University Line pp113-124.
3. Stura E.A, Satterthwait, A.C, Calvo, J.C, Kaslow, D.C, Wilson, I.A. (1994) Reverse Screening. Acta Cryst. D50 : 448-455.
4. Stura E.A, at the "EMBO Workshop on the crystallization of Macromolecular Complexes" Grenoble 8-13 April 2001

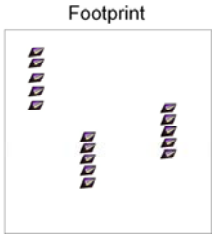


MacroSol – Set 1

	1	2	3	4	5	6
A	10% v/v MPD 0.02 M calcium chloride	1.0 M sodium acetate 0.1M NaH_2PO4	8% w/v PEG 3350 0.2 M ammonium acetate	0.75 M ammonium phosphate	10% v/v 2-propanol 0.2 M magnesium chloride	16% w/v PEG 3350 0.2 M lithium sulfate
B	20% v/v MPD 0.02 M calcium chloride	1.4 M sodium acetate 0.1 M NaH_2PO4	15% w/v PEG 3350 0.2 M ammonium acetate	1.0 M ammonium phosphate	15% v/v 2-propanol 0.2 M magnesium chloride	25% w/v PEG 3350 0.2 M lithium sulfate
C	30% v/v MPD	1.7 M sodium acetate 0.1 M NaH_2PO4	20% w/v PEG 3350 0.2 M ammonium acetate	1.5 M ammonium phosphate	20% v/v 2-propanol 0.2 M magnesium chloride	30% w/v PEG 3350 0.2 M lithium sulfate
D	30% v/v MPD 0.02 M calcium chloride	1.7 M sodium acetate	30% w/v PEG 3350 0.2 M ammonium acetate	2.0 M ammonium phosphate	30% v/v 2-propanol 0.2 M magnesium chloride	25% w/v PEG 3350 0.5 M lithium sulfate
Buffer	**0.1M Sodium acetate pH 4.5**	**0.1M Sodium cacodylate pH 6.5**	**0.1M Sodium acetate pH 4.5**	**0.1M Sodium citrate pH 5.5**	**0.1M HEPES pH 7.5**	**0.1M Tris HCl pH 8.5**

MacroSol – Set 2

	1	2	3	4	5	6
A	2% w/v PEG 8000 1.0 M lithium sulfate	12% w/v PEG 8000 0.2 M ammonium sulfate	10% w/v PEG 4000 0.5 M ammonium sulfate	2% v/v PEG 400 1.0 M ammonium sulfate	12% w/v PEG 4000 5% v/v 2-propanol	9% w/v PEG 8000 0.005 M zinc acetate
B	2% w/v PEG 8000 1.0 M lithium sulfate 0.1M Buffer pH 5.5	18% w/v PEG 8000 0.2 M ammonium sulfate	20% w/v PEG 4000 0.3 M ammonium sulfate	1.5 M ammonium sulfate 2% v/v PEG 400	16% w/v PEG 4000 10% v/v 2-propanol	12% w/v PEG 8000 0.005 M zinc acetate
C	2% w/v PEG 8000 1.0M Lithium sulfate 0.1M Buffer pH 6.5	24% w/v PEG 8000 0.2 M ammonium sulfate	30% w/v PEG 4000 0.2 M ammonium sulfate	2.0 M ammonium sulfate 2% v/v PEG 400	20% w/v PEG 4000 15% v/v 2-propanol	18% w/v PEG 8000 0.005 M zinc acetate
D	2% w/v PEG 8000 1.0 M lithium sulfate 0.1M Buffer pH 7.5	30% w/v PEG 8000 0.2 M ammonium sulfate	36% w/v PEG 4000 0.2 M ammonium sulfate	5% v/v PEG 400 2.0 M ammonium sulfate	20% w/v PEG 4000 20% v/v 2-propanol	24% w/v PEG 8000 0.005 M zinc acetate
Buffer	**0.1 M imidazole Malate**	**None**	**None**	**0.1 M Hepes pH 7.5**	**0.1 M sodium citrate pH 5.5**	**0.1 M sodium cacodylate pH 6.5**



MacroSol MD1-22

Tube Set	Reagent 1	Reagent 2	Buffer	pH	Position
1-1	10% v/v MPD	0.02 M calcium chloride	0.1 M sodium acetate	4.5	1A
1-2	20% v/v MPD	0.02 M calcium chloride	0.1 M sodium acetate	4.5	1B
1-3	30% v/v MPD	-	0.1 M sodium acetate	4.5	1C
1-4	30% v/v MPD	0.02 M calcium chloride	0.1 M sodium acetate	4.5	1D
1-5	1.0 M sodium acetate	0.1 M sodium dihydrogen phosphate	0.1 M sodium cacodylate	6.5	2A
1-6	1.4 M sodium acetate	0.1 M sodium dihydrogen phosphate	0.1 M sodium cacodylate	6.5	2B
1-7	1.7 M sodium acetate	0.1 M sodium dihydrogen phosphate	0.1 M sodium cacodylate	6.5	2C
1-8	1.7 M sodium acetate	-	0.1 M sodium cacodylate	6.5	2D
1-9	8% w/v PEG 3350	0.2 M ammonium acetate	0.1 M sodium acetate	4.5	3A
1-10	15% w/v PEG 3350	0.2 M ammonium acetate	0.1 M sodium acetate	4.5	3B
1-11	20% w/v PEG 3350	0.2 M ammonium acetate	0.1 M sodium acetate	4.5	3C
1-12	30% w/v PEG 3350	0.2 M ammonium acetate	0.1 M sodium acetate	4.5	3D
1-13	0.75 M ammonium dihydrogen phosphate	-	0.1 M sodium citrate	5.5	4A
1-14	1.0 M ammonium dihydrogen phosphate	-	0.1 M sodium citrate	5.5	4B
1-15	1.5 M ammonium dihydrogen phosphate	-	0.1 M sodium citrate	5.5	4C
1-16	2.0 M ammonium dihydrogen phosphate	-	0.1 M sodium citrate	5.5	4D
1-17	10% v/v 2-propanol	0.2 M magnesium chloride	0.1 M Na HEPES	7.5	5A
1-18	15% v/v 2-propanol	0.2 M magnesium chloride	0.1 M Na HEPES	7.5	5B
1-19	20% v/v 2-propanol	0.2 M magnesium chloride	0.1 M Na HEPES	7.5	5C
1-20	30% v/v 2-propanol	0.2 M magnesium chloride	0.1 M Na HEPES	7.5	5D
1-21	16% w/v PEG 3350	0.2 M lithium sulfate	0.1 M Tris	8.5	6A
1-22	25% w/v PEG 3350	0.2 M lithium sulfate	0.1 M Tris	8.5	6B
1-23	30% w/v PEG 3350	0.2 M lithium sulfate	0.1 M Tris	8.5	6C
1-24	25% w/v PEG 3350	0.5 M lithium sulfate	0.1 M Tris	8.5	6D
2-1	2% w/v PEG 8000	1.0 M lithium sulfate	-		1A
2-2	2% w/v PEG 8000	1.0 M lithium sulfate	0.1 M imidazole malate	5.5	1B
2-3	2% w/v PEG 8000	1.0 M lithium sulfate	0.1 M imidazole malate	6.5	1C
2-4	2% w/v PEG 8000	1.0 M lithium sulfate	0.1 M imidazole malate	7.5	1D
2-5	12% w/v PEG 8000	0.2 M ammonium sulfate	-	-	2A
2-6	18% w/v PEG 8000	0.2 M ammonium sulfate	-	-	2B
2-7	24% w/v PEG 8000	0.2 M ammonium sulfate	-	-	2C
2-8	30% w/v PEG 8000	0.2 M ammonium sulfate	-	-	2D
2-9	10% w/v PEG 4000	0.5 M ammonium sulfate	-	-	3A
2-10	20% w/v PEG 4000	0.3 M ammonium sulfate	-	-	3B
2-11	30% w/v PEG 4000	0.2 M ammonium sulfate	-	-	3C
2-12	36% w/v PEG 4000	0.2 M ammonium sulfate	-	-	3D
2-13	1.0 M ammonium sulfate	2% v/v PEG 400	0.1 M Na HEPES	7.5	4A
2-14	1.5 M ammonium sulfate	2% v/v PEG 400	0.1 M Na HEPES	7.5	4B
2-15	2.0 M ammonium sulfate	2% v/v PEG 400	0.1 M Na HEPES	7.5	4C
2-16	2.0 M ammonium sulfate	5% v/v PEG 400	0.1 M Na HEPES	7.5	4D
2-17	12% w/v PEG 4000	5% v/v 2-propanol	0.1 M sodium citrate	5.5	5A
2-18	16% w/v PEG 4000	10% v/v 2-propanol	0.1 M sodium citrate	5.5	5B
2-19	20% w/v PEG 4000	15% v/v 2-propanol	0.1 M sodium citrate	5.5	5C
2-20	20% w/v PEG 4000	20% v/v 2-propanol	0.1 M sodium citrate	5.5	5D
2-21	9% w/v PEG 8000	0.005 M zinc acetate	0.1 M sodium cacodylate	6.5	6A
2-22	12% w/v PEG 8000	0.005 M zinc acetate	0.1 M sodium cacodylate	6.5	6B
2-23	18% w/v PEG 8000	0.005 M zinc acetate	0.1 M sodium cacodylate	6.5	6C
2-24	24% w/v PEG 8000	0.005 M zinc acetate	0.1 M sodium cacodylate	6.5	6D

Abbreviations: Na HEPES; 2-(4-(2-Hydroxyethyl)-1-piperazinyl)ethanesulfonic Acid Sodium Salt, **PEG**; Polyethylene glycol, **Tris**; 2-Amino-2-(hydroxymethyl) propane-1,3-diol. Note: The pH of each final reagent is checked and adjusted back to the stated pH of the buffer (±0.2 pH units) as appropriate.

Manufacturer's datasheets are available on request