Molecular Dimensions

MD1-103

Protein Stability Combo Kit

(MD1-96, MD1-97, & MD1-101, MD1-102)

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Molecular Dimensions

RUBIC Buffer Screen MD1-96

For stable, happy proteins – From purification all the way through to characterization by NMR, SAXS or Crystallography.

RUBIC Buffer Screen- designed at the EMBL Hamburg and optimized for Differential Scanning Fluorimetry/ThermoFluor and Thermal Stability Assays to determine optimum conditions for protein stability, purification and storage.

MD1-96 is presented as 96 x 0.5 mL conditions in a deep-well block.

Features of RUBIC Buffer Screen:

- Conditions optimized for Differential Scanning Fluorimetry (DSF).
- Identify conditions that enhance protein stability.
- Optimize purification and storage conditions.
- Screen for global parameters e.g. pH, salt concentrations, buffer type and concentration.
- Tested on more than 200 different protein constructs.
- Suits a wide range of proteins (small, large, complex, DNA binding proteins etc.) and applications.
- Compatible with Thermofluor and Protein Thermal Shift assays.

Introduction

RUBIC Buffer Screen is a set of 96 chemical reagents formulated in ultrapure water at room temperature. Conditions are optimized for Differential Scanning Fluorimetry (DSF) assay to identify solution conditions which enhance protein stability and to optimize purification and storage protocols. RUBIC Buffer Screen has been created in such a way, that it is possible to discern global stability trends according to:

- pH
- salt concentration
- buffer type
- buffer concentration

Storage

RUBIC Buffer Screen is free of preservatives. Shipping at Room Temperature. Short-term Storage at 4˚C. It is recommended that users prefill plates and store them at -20˚C.

Differential Scanning Fluorimetry (DSF) assay approach

DSF takes advantage of the fact that the fluorescence of many nonspecific protein-binding dyes (e.g. SYPRO Orange) increases together with increasing hydrophobicity of their environment. In principle, the protein solution is heated in the presence of SYPRO Orange. Upon denaturation, the dye binds to the internal hydrophobic protein core increasing the fluorescence significantly. Maximal fluorescence signal is obtained when the protein unfolds completely, then the SYPRO Orange signal decreases corresponding to dye-protein dissociation. The fluorescence signal is plotted as a function of temperature to get a sigmoidal curve that shows the fraction of the unfolded protein. The inflection point corresponds to the melting temperature (*Tm*), at which 50% of the protein is unfolded (Fig. 1).

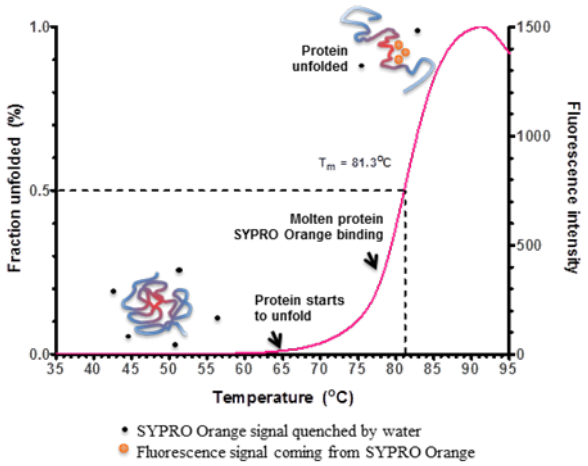


Figure 1

Fig. 1. Typical thermal denaturation assay using DSF. Figure adapted from Boivin et al., 2013

Sample requirement:

- ~200 µl of protein in a low ionic sample buffer free of stabilizing reagent. Initial protein at ~20 µM (35 kDa) is normally sufficient to visualize a melting curve with a good signal-to-noise ratio. Lower concentration can be used with protein of higher molecular weight, while low molecular weight proteins may require a higher concentration.

- Sample buffer should contain reagent to stabilize protein, we recommend not to exceed NaCl (<200 mM), glycerol (<10 %), reducing reagent (<5 mM).

- Assay is not compatible with most detergents.

Suggested protocol:

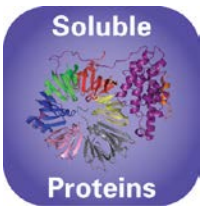
1. Transfer 21 µl of RUBIC Buffer Screen to a PCR-microplate.
2. Spin down the prefilled microplate for 30 seconds.
3. Place the microplate on ice.
4. Dispense in each well 2 µl of the protein. It is strongly advised to use a repeater pipette.
5. Prepare freshly a SYPRO Orange solution (Invitrogen, S6651, 5000X) at 62X by diluting 3 µl of 5000X stock in 237 µl of water. 240 µl is sufficient to test 96 conditions.
6. Dispense in each well 2 µl of diluted SYPRO Orange solution. The final working concentration will be 5X.
7. Seal the PCR-plate with ClearVue Sheets or clear adhesive seal.
8. Spin down the PCR-plate for 30 seconds.
9. Place the microplate in the RT-PCR machine pre-equilibrated at the desired temperature. We recommend using a temperature gradient of 1°C/min from 5 or 20 to 95°C. Make sure to use a pair of filters compatible with the maximum excitation and emission wavelengths of SYPRO Orange (i.e. SybrGreen).

Note: We advise against pre-mixing the protein and the dye. Since the dye contains DMSO, it can damage the protein in higher concentrations or interact with the protein affecting the initial background signal.

Data analysis

The analysis of DSF data is based on a plot of the melting curve that represents relative values of the detected fluorescence intensity. To identify a buffer condition that stabilizes the protein, the *Tm* value of the protein under each condition of the RUBIC Buffer Screen needs to be compared with the reference *Tm*. To simplify the analysis we recommend organizing the data by categories such as:

- buffer type and salt effect (A1-B12; C1-D12)
- pH effect (E1-E12)
- buffer concentration effect (F1-F4; F5-F8;F9-12)
- salt concentration effect (G1-G6; G7-G12)
- buffer systems (H1-H7)
- imidazole (H8-H12)



Formulation Notes:

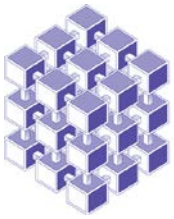
RUBIC Buffer Screen reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added. Prepared at room temperature.

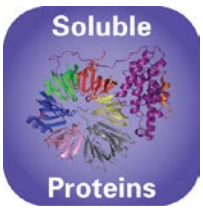
Final pH may vary from that specified on the datasheet. Molecular Dimensions will be happy to discuss the precise formulation of individual reagents. Individual reagents and stock solutions for optimization are available from Molecular Dimensions.

Contact and product details can be found at www.moleculardimensions.com
Enquiries regarding RUBIC Buffer Screen formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

References

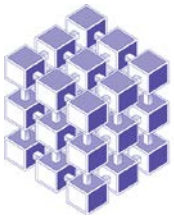
Boivin S, Kozak S, Meijers R. (2013) *Optimization of protein purification and characterization using Thermofluor screens.* Protein Expr Purif. 91(2):192-206.
Newman J. (2004) *Novel buffer systems for macromolecular crystallization.* Acta Crystallogr D Biol Crystallogr. 60:610-2.

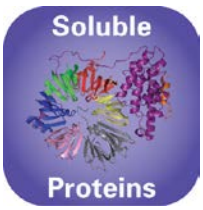




RUBIC Buffer Screen Conditions A1-D12* MD1-96

Well No.	Conc.	Units	Reagent	Conc	Units	Buffer	pH
A1	100	%	Ultrapure water				
A2				0.119	M	Citrate	4.0
A3				0.119	M	Sodium acetate	4.5
A4				0.119	M	Citrate	5.0
A5				0.119	M	MES	6.0
A6				0.119	M	Potassium phosphate	6.0
A7				0.119	M	Citrate	6.0
A8				0.119	M	Bis-Tris	6.5
A9				0.119	M	MES	6.5
A10				0.119	M	Sodium phosphate	7.0
A11				0.119	M	Potassium phosphate	7.0
A12				0.119	M	HEPES	7.0
B1				0.119	M	MOPS	7.0
B2				0.119	M	Ammonium acetate	7.3
B3				0.119	M	Tris-HCl	7.5
B4				0.119	M	Sodium phosphate	7.5
B5				0.119	M	Imidazole	7.5
B6				0.119	M	HEPES	8.0
B7				0.119	M	Tris-HCl	8.0
B8				0.119	M	Tricine	8.0
B9				0.119	M	BICINE	8.0
B10				0.119	M	BICINE	8.5
B11				0.119	M	Tris-HCl	8.5
B12				0.119	M	CHES	9.0
C1	0.298	M	Sodium chloride				
C2	0.298	M	Sodium chloride	0.119	M	Citrate	4.0
C3	0.298	M	Sodium chloride	0.119	M	Sodium acetate	4.5
C4	0.298	M	Sodium chloride	0.119	M	Citrate	5.0
C5	0.298	M	Sodium chloride	0.119	M	MES	6.0
C6	0.298	M	Sodium chloride	0.119	M	Potassium phosphate	6.0
C7	0.298	M	Sodium chloride	0.119	M	Citrate	6.5
C8	0.298	M	Sodium chloride	0.119	M	Bis-Tris	6.5
C9	0.298	M	Sodium chloride	0.119	M	MES	6.5
C10	0.298	M	Sodium chloride	0.119	M	Sodium phosphate	7.0
C11	0.298	M	Sodium chloride	0.119	M	Potassium phosphate	7.0
C12	0.298	M	Sodium chloride	0.119	M	CHES	7.0
D1	0.298	M	Sodium chloride	0.119	M	MOPS	7.0
D2	0.298	M	Sodium chloride	0.119	M	Ammonium acetate	7.3
D3	0.298	M	Sodium chloride	0.119	M	Tris-HCl	7.5
D4	0.298	M	Sodium chloride	0.119	M	Sodium phosphate	7.5
D5	0.298	M	Sodium chloride	0.119	M	Imidazole	8.0
D6	0.298	M	Sodium chloride	0.119	M	HEPES	8.0
D7	0.298	M	Sodium chloride	0.119	M	Tris-HCl	8.0
D8	0.298	M	Sodium chloride	0.119	M	Tricine	8.0
D9	0.298	M	Sodium chloride	0.119	M	BICINE	8.0
D10	0.298	M	Sodium chloride	0.119	M	BICINE	8.5
D11	0.298	M	Sodium chloride	0.119	M	Tris-HCl	8.5
D12	0.298	M	Sodium chloride	0.119	M	CHES	9.0





RUBIC Buffer Screen Conditions E1-H12* MD1-96

Well No.	Conc.	Units	Reagent	Conc	Units	Buffer	pH
E1				0.119	M	SPG	4.0
E2				0.119	M	SPG	4.5
E3				0.119	M	SPG	5.0
E4				0.119	M	SPG	5.5
E5				0.119	M	SPG	6.0
E6				0.119	M	SPG	6.5
E7				0.119	M	SPG	7.0
E8				0.119	M	SPG	7.5
E9				0.119	M	SPG	8.0
E10				0.119	M	SPG	8.5
E11				0.119	M	SPG	9.0
E12				0.119	M	SPG	10.0
F1				0.024	M	HEPES	7.5
F2				0.06	M	HEPES	7.5
F3				0.149	M	HEPES	7.5
F4				0.298	M	HEPES	7.5
F5				0.024	M	Sodium phosphate	7.5
F6				0.06	M	Sodium phosphate	7.5
F7				0.149	M	Sodium phosphate	7.5
F8				0.298	M	Sodium phosphate	7.5
F9				0.024	M	Tris-HCl	8.0
F10				0.06	M	Tris-HCl	8.0
F11				0.149	M	Tris-HCl	8.0
F12				0.298	M	Tris-HCl	8.0
G1	0.06	M	Sodium chloride	0.06	M	HEPES	7.5
G2	0.149	M	Sodium chloride	0.06	M	HEPES	7.5
G3	0.298	M	Sodium chloride	0.06	M	HEPES	7.5
G4	0.595	M	Sodium chloride	0.06	M	HEPES	7.5
G5	0.893	M	Sodium chloride	0.06	M	HEPES	7.5
G6	1.19	M	Sodium chloride	0.06	M	HEPES	7.5
G7	0.06	M	Sodium chloride	0.06	M	Tris-HCl	8.0
G8	0.149	M	Sodium chloride	0.06	M	Tris-HCl	8.0
G9	0.298	M	Sodium chloride	0.06	M	Tris-HCl	8.0
G10	0.595	M	Sodium chloride	0.06	M	Tris-HCl	8.0
G11	0.893	M	Sodium chloride	0.06	M	Tris-HCl	8.0
G12	1.19	M	Sodium chloride	0.06	M	Tris-HCl	8.0
H1				0.06	M	MES/Bis-Tris	6.0
H2				0.06	M	MES/Imidazole	6.5
H3				0.06	M	Bis-Tris/PIPES	6.5
H4				0.06	M	MOPS/Bis-Tris propane	7.0
H5				0.06	M	Phosphate/Citrate	7.5
H6				0.06	M	MOPS/Sodium HEPES	7.5
H7				0.06	M	BICINE/Tris	8.5
H8	0.119	M	Sodium chloride	0.06	M	Imidazole	7.5
H9	0.119	M	Sodium chloride	0.149	M	Imidazole	7.5
H10	0.119	M	Sodium chloride	0.298	M	Imidazole	7.5
H11	0.119	M	Sodium chloride	0.417	M	Imidazole	7.5
H12	0.119	M	Sodium chloride	0.595	M	Imidazole	7.5

***concentrations shown are not final concentrations. For the final concentrations- see Figure 2**

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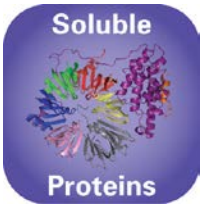
Soluble Proteins

Figure 2:- Layout of the of RUBIC Buffer Screen

	1	2	3	4	5	6	7	8	9	10	11	12	
A	Water	100mM Citric Acid pH 4.0	100mM NaAcetate pH 4.5	100mM Citric Acid pH 5.0	100mM MES pH 6.0	100mM KPhosphate (monobasic) pH 6.0	100mM Citric Acid pH 6.0	100mM Bis-Tris pH 6.5	100mM Mes pH 6.5	100mM Na2Phosphate (dibasic) pH 7.0	100mM KPhosphate (monobasic) pH 7.0	100mM HEPES pH 7.0	Buffer and pH screens (low ionic strength)
B	100mM MOPS pH 7.0	100mM AmAcetate pH 7.3	100mM Tris-HCl pH 7.5	100mM Na2Phosphate (dibasic) pH 7.5	100mM Imidazole pH 7.5	100mM HEPES ph 8.0	100mM Tris-HCl pH 8.0	100mM Tricine pH 8.0	100mM Bicine pH 8.0	100mM Bicine pH 8.5	100mM Tris-HCl pH 8.5	100mM CHES pH 9.0	
C	Water 250mM NaCl	100mM Citric Acid 250mM NaCl pH 4.0	100mM NaAcetate 250mM NaCl pH 4.5	100mM Citric Acide 250mM NaCl pH 5.0	100mM MES 250mM NaCl pH 6.0	100mM KPhosphate 250mM NaCl pH 6.0	100mM Citric Acid 250mM NaCl pH 6.0	100mM Bis-Tris 250mM NaCl pH 6.5	100mM Mes 250mM NaCl pH 6.5	100mM Na2Phosphate 250mM NaCl pH 7.0	100mM KPhosphate 250mM NaCl pH 7.0	100mM HEPES 250mM NaCl pH 7.0	Buffer and pH screens (high ionic strength)
D	100mM MOPS 250mM NaCl pH 7.0	100mM AmAcetate 250mM NaCl pH 7.3	100mM Tris-HCl 250mM NaCl pH 7.5	100mM Na2Phosphate 250mM NaCl pH 7.5	100mM Imidazol 250mM NaCl pH 7.5	100mM HEPES 250mM NaCl ph 8.0	100mM Tris-HCl 250mM NaCl pH 8.0	100mM Tricine 250mM NaCl pH 8.0	100mM Bicine 250mM NaCl pH 8.0	100mM Bicine 250mM NaCl pH 8.5	100mM Tris-HCl 250mM NaCl pH 8.5	100mM CHES 250mM NaCl pH 9.0	
E	100mM SPG pH 4.0	100mM SPG pH 4.5	100mM SPG pH 5.0	100mM SPG pH 5.5	100mM SPG pH 6.0	100mM SPG pH 6.5	100mM SPG pH 7.0	100mM SPG pH 7.5	100mM SPG pH 8.0	100mM SPG pH 8.5	100mM SPG pH 9.0	100mM SPG pH 10.0	Extended range pH buffer (deconvolute pH from buffer effect)
F	20mM HEPES pH 7.5	50mM HEPES pH 7.5	125mM HEPES pH 7.5	250mM HEPES pH 7.5	20mM Na2Phosphate (dibasic) pH 7.5	50mM Na2Phosphate (dibasic) pH 7.5	125mM Na2Phosphate (dibasic) pH 7.5	250mM Na2Phosphate (dibasic) pH 7.5	20mM Tris-HCl pH 8.0	50mM Tris-HCl pH 8.0	125mM Tris-HCl pH 8.0	250mM Tris-HCl pH 8.0	Ionic strength effect (Buffer)
G	50mM HEPES 50mM NaCl pH 7.5	50mM HEPES 125mM NaCl pH 7.5	50mM HEPES 250mM NaCl pH 7.5	50mM HEPES 500mM NaCl pH 7.5	50mM HEPES 750mM NaCl pH 7.5	50mM HEPES 1000mM NaCl pH 7.5	50mM Tris-HCl 50mM NaCl pH 8.0	50mM Tris-HCl 125mM NaCl pH 8.0	50mM Tris-HCl 250mM NaCl pH 8.0	50mM Tris-HCl 500mM NaCl pH 8.0	50mM Tris-HCl 750mM NaCl pH 8.0	50mM Tris-HCl 1000mM NaCl pH 8.0	Ionic strength effect (Salt)
H	50mM MES / Bis-Tris pH 6.0	50mM MES / imidazole pH 6.5	50mM Bis-Tris / PIPES pH 6.5	50mM MOPS / Bis-Tris propane pH 7.0	50mM NaPhosphate / citric acid pH 7.5	50mM MOPS / HEPES-Na pH 7.5	0.1M Bicine / Trizma base pH 8.5	50mM Imidazole 100mM NaCl pH 7.5	125mM Imidazole 100mM NaCl pH 7.5	250mM Imidazole 100mM NaCl pH 7.5	350mM Imidazole 100mM NaCl pH 7.5	500mM Imidazole 100mM NaCl pH 7.5	Buffer Systems Imidazole

Concentrations shown are final concentration based on 25 µl assay
(21 µL RUBIC Buffer Screen + 2 µL Protein sample + 2 µL SYPRO Orange dye diluted stock solution).



Abbreviations:
SPG: Succinic acid/sodium Phosphate monobasic/Glycine [2:7:7].

Manufacturer's safety data sheets are available from our website.

Re-Ordering Details:

Catalogue Description	Pack size	Catalogue Code
RUBIC Buffer Screen	96 x 0.5 mL	MD1-96
RUBIC Additive Screen	96 x 0.25 mL	MD1-97
RUBIC Buffer Set*	48 x 11 mL	MD1-96-BUFFER
Single Reagents		
RUBIC Buffer Screen single reagents	10 mL	MDSR-96-well number
RUBIC Additive Screen single reagents	various volumes	See website for more details.

All stocks are available to buy from Molecular Dimensions.

*The RUBIC Buffer Set contains buffers A1 to B24 at 0.5M Buffer, C1 to D24 at 0.5M Buffer+ 1.25M NaCl from the RUBIC Buffer Screen.

Molecular Dimensions

RUBIC Additive Screen MD1-97

For stable, happy proteins – From purification all the way through to characterization by NMR, SAXS or Crystallography.

RUBIC Additive Screen - screen a wide-range of small molecules and increase protein stability by selecting a buffer, additives and ligands compatible with your protein of interest.

MD1-97 is presented as 96 x 0.25* mL conditions and 24 x 1.5mL 5X (500 mM) buffers
(*enough for 15 experiments)

Features of RUBIC Additive Screen

- Wide-range of additives: salts, monovalent and multivalent ions, chaotropic reagents, NDSB's, detergents, carbohydrates, carboxylic acids, amino acids, polyols, reducing agents, linkers, co-factors, polyamines and ligands.
- Use from protein purification all the way through to characterization by NMR, SAXS or X-Ray.
- Great versatility- allows customisation of buffer compatible with protein of interest.
- Use as a silver bullet.

Introduction

The Additive Screen contains small molecules that can affect the folding, aggregation state and solubility of the protein, and also includes small molecules that specifically bind and stabilize proteins. The Additive Screen consists of a selection of different physiological and non-physiological ligands that include amino acids, nucleotides, sugars, cofactors, monovalent and divalent ions, and some other additives.

Ligand-induced conformational stabilization of proteins is a well-understood phenomenon. Substrates, inhibitors, cofactors, and protein binding partners provide enhanced stability to proteins by selective binding. A thermal denaturation assay can be used to screen for the effect of additives while the buffer conditions are kept constant. Upon ligand binding, the protein complex denatures at a higher temperature and the difference in the Tm value in the presence and absence of the compound reflects ligand binding.

Storage

RUBIC Additive Screen is free of preservatives. Shipping is on ice. Product may thaw during shipping; this will in no way affect its use. It is recommended that users prefill plates and store them at -20˚C or -80 ˚C if possible upon receipt.

Thus, the thermal shift assay can serve as a tool to search for stabilizing reagents, a 'silver bullet' for the crystallization of proteins and to identify natural ligands that provide insight into the biological function of the protein.

Differential Scanning Fluorimetry (DSF) assay approach

DSF takes advantage of the fact that the fluorescence of many nonspecific protein-binding dyes (e.g. SYPRO Orange) increases together with increasing hydrophobicity of their environment. In principle, the protein solution is heated in the presence of SYPRO Orange. Upon denaturation, the dye binds to the internal hydrophobic protein core increasing significantly the fluorescence. Maximal fluorescence signal is obtained when the protein unfolds completely, then SYPRO Orange signal decreases corresponding to dye-protein dissociation. The fluorescence signal is plotted as a function of temperature to get a sigmoidal curve that shows the fraction of the unfolded protein. The inflection point corresponds to the melting temperature (*Tm*), at which 50% of the protein is unfolded (Figure. 1).

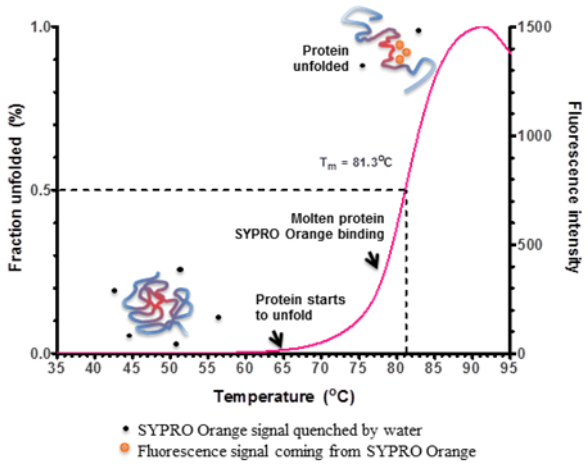
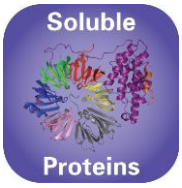


Figure 1

Figure. 1. Typical thermal denaturation assay using Thermofluor.



Sample requirement:

- ~210µl of protein in low ionic sample buffer free of stabilizing reagent. Initial protein at 10 - 20µM (35kDa) is normally sufficient to visualize a melting curve with a good signal-to-noise ratio. Lower concentration can be used with larger proteins or complexes, while smaller proteins may require a more concentrated sample.

- Sample buffer should contain reagent to stabilize protein, we recommend not to exceed NaCl (<200uM), glycerol (<10%), reducing reagent (<5mM).

Suggested protocol:

1. Thaw the RUBIC Additive Screen on ice. (Avoid multiple freeze-thaw cycles).
2. Spin down the prefilled microplate for 30 seconds.
3. Transfer 16 µl of RUBIC Additive Screen to a PCR-microplate.
4. Add 5 µl of a 5X buffer. We advise to use a buffer free of salt to prevent competition with reagent from the additive screen. Several 5X ready-to-use buffers are provided with the screen (See Table 1).
5. Dispense 2 µl of protein sample into each well. It is strongly advised to use a repeater pipette.
6. Freshly prepare a SYPRO Orange solution at 62X by diluting 3 µl of 5000X stock in 237 µl of water (Invitrogen, S6651, 5000X). 240 µl is sufficient to test 96 conditions.
7. Dispense 2 µl of diluted SYPRO Orange solution into each well. The final working concentration will be 5X.
8. Seal the PCR-plate with a clear adhesive seal (e.g. ClearVue Sheets MD6-01S).
9. Spin down the PCR-plate for 30 seconds.
10. Place the microplate in the RT-PCR machine pre-equilibrated at the desired temperature. We recommend using a temperature gradient of 1°C/min from 5 or 20 to 95°C. The pair of filters (i.e. SYBRGreen) should be compatible with the maximum excitation and emission wavelengths of SYPRO Orange that is 470 and 569 nm, respectively.

Note: We advise against pre-mixing the protein and the dye. Since the dye contains DMSO, it can damage the protein in higher concentrations or interact with the protein affecting the initial background signal.

Data analysis

The analysis of Thermofluor data is based on a plot of the melting curve that represents relative values of the detected fluorescence intensity. To identify an additive that stabilizes the protein, the *Tm* value of the protein under each condition of the RUBIC Additive Screen needs to be compared with the reference *Tm*. To simplify the analysis we recommend organizing the data by categories such as:

- **Salts (A1-B7)**
- **Monovalent ions (B8-C5)**
- **Multivalent ions (C6-D2)**
- **Chaotropic reagents (D3-D9)**
- **Non-detergent sulfobetaines, detergents (D10-E5)**
- **Carbohydrates (E6-E9)**
- **Carboxylic acids, amino acids (E10-F8)**
- **Polyols (F9-G3)**
- **Reducing reagents (G4-G5)**
- **Co-factors, polyamines, Ligands (G6-G12)**
- **Nucleotides (H1-H10)**
- **Imidazole (H11-H12)**

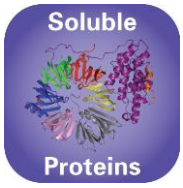


Table 1. Buffers contained in the RUBIC Additive Kit (Tube 1 x 1.5mL water and 23 x 1.5mL buffers).

Tube No.	Conc.	Units	Reagent	pH
1	100	%	Ultrapure water	
2	500	mM	Citrate	4.0
3	500	mM	Sodium acetate	4.5
4	500	mM	Citrate	5.0
5	500	mM	MES	6.0
6	500	mM	Potassium phosphate	6.0
7	500	mM	Citrate	6.0
8	500	mM	Bis-Tris	6.5
9	500	mM	MES	6.5
10	500	mM	Sodium phosphate	7.0
11	500	mM	Potassium phosphate	7.0
12	500	mM	HEPES	7.0
13	500	mM	MOPS	7.0
14	500	mM	Ammonium acetate	7.3
15	500	mM	Tris-HCl	7.5
16	500	mM	Sodium phosphate	7.5
17	500	mM	Imidazole	7.5
18	500	mM	HEPES	8.0
19	500	mM	Tris-HCl	8.0
20	500	mM	Tricine	8.0
21	500	mM	BICINE	8.0
22	500	mM	BICINE	8.5
23	500	mM	Tris-HCl	8.5
24	500	mM	CHES	9.0

Formulation Notes:

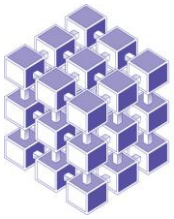
RUBIC Additive Screen reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 μm filters. No preservatives are added.

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 optimization are available from Molecular Dimensions.

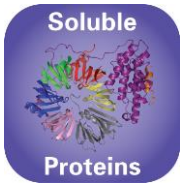
Contact and product details can be found at www.moleculardimensions.com
Enquiries regarding RUBIC Additive Screen formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

References

Boivin S, Kozak S, Meijers R. (2013) *Optimization of protein purification and characterization using Thermofluor screens.* Protein Expr Purif. 91(2):192-206.
Newman J. (2004) *Novel buffer systems for macromolecular crystallization.* Acta Crystallogr D Biol Crystallogr. 60:610-2.

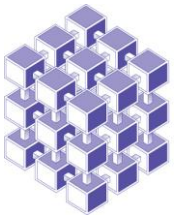


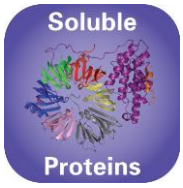
Molecular Dimensions



RUBIC Additive Screen Conditions A1-D12 MD1-97

Number	Position	Prefilled microplate (16 µL)		Final concentration during assay (25 µL)	
1	A01	100 %	Ultrapure water	100 %	Ultrapure water
2	A02	156 mM	Sodium acetate trihydrate	100 mM	Sodium acetate trihydrate
3	A03	156 mM	Calcium acetate hydrate	100 mM	Calcium acetate hydrate
4	A04	156 mM	Potassium acetate	100 mM	Potassium acetate
5	A05	156 mM	Ammonium acetate	100 mM	Ammonium acetate
6	A06	156 mM	Sodium sulfate	100 mM	Sodium sulfate
7	A07	156 mM	Magnesium sulfate heptahydrate	100 mM	Magnesium sulfate heptahydrate
8	A08	156 mM	Potassium sulfate	100 mM	Potassium sulfate
9	A09	156 mM	Ammonium sulfate	100 mM	Ammonium sulfate
10	A10	156 mM	Sodium phosphate monobasic monohydrate	100 mM	Sodium phosphate monobasic monohydrate
11	A11	156 mM	Sodium phosphate dibasic	100 mM	Sodium phosphate dibasic
12	A12	156 mM	Potassium phosphate monobasic	100 mM	Potassium phosphate monobasic
13	B01	156 mM	Potassium phosphate dibasic	100 mM	Potassium phosphate dibasic
14	B02	156 mM	Sodium tartrate dibasic dihydrate	100 mM	Sodium tartrate dibasic dihydrate
15	B03	156 mM	Sodium citrate tribasic dihydrate	100 mM	Sodium citrate tribasic dihydrate
16	B04	156 mM	Sodium malonate dibasic monohydrate	100 mM	Sodium malonate dibasic monohydrate
17	B05	156 mM	Sodium nitrate	100 mM	Sodium nitrate
18	B06	156 mM	Sodium formate	100 mM	Sodium formate
19	B07	156 mM	Potassium formate	100 mM	Potassium formate
20	B08	156 mM	Sodium fluoride	100 mM	Sodium fluoride
21	B09	156 mM	Potassium fluoride	100 mM	Potassium fluoride
22	B10	156 mM	Ammonium fluoride	100 mM	Ammonium fluoride
23	B11	156 mM	Lithium chloride	100 mM	Lithium chloride
24	B12	156 mM	Sodium chloride	100 mM	Sodium chloride
25	C01	156 mM	Potassium chloride	100 mM	Potassium chloride
26	C02	156 mM	Ammonium chloride	100 mM	Ammonium chloride
27	C03	156 mM	Sodium iodide	100 mM	Sodium iodide
28	C04	156 mM	Potassium iodide	100 mM	Potassium iodide
29	C05	156 mM	Sodium bromide	100 mM	Sodium bromide
30	C06	1.56 mM	Magnesium chloride hexahydrate	1 mM	Magnesium chloride hexahydrate
31	C07	1.56 mM	Calcium chloride dihydrate	1 mM	Calcium chloride dihydrate
32	C08	1.56 mM	Manganese(II) chloride tetrahydrate	1 mM	Manganese(II) chloride tetrahydrate
33	C09	1.56 mM	Nickel(II) chloride hexahydrate	1 mM	Nickel(II) chloride hexahydrate
34	C10	1.56 mM	Iron(III) chloride hexahydrate	1 mM	Iron(III) chloride hexahydrate
35	C11	1.56 mM	Zinc chloride	1 mM	Zinc chloride
36	C12	1.56 mM	Cobalt(II) chloride hexahydrate	1 mM	Cobalt(II) chloride hexahydrate
37	D01	7.81 mM	EDTA	5 mM	EDTA
38	D02	7.81 mM	EGTA	5 mM	EGTA
39	D03	0.16 M	Urea	0.1 M	Urea
40	D04	0.78 M	Urea	0.5 M	Urea
41	D05	1.56 M	Urea	1 M	Urea
42	D06	3.12 M	Urea	2 M	Urea
43	D07	6.25 M	Urea	4 M	Urea
44	D08	234 mM	Guanidine hydrochloride	150 mM	Guanidine hydrochloride
45	D09	781 mM	Guanidine hydrochloride	500 mM	Guanidine hydrochloride
46	D10	1.56 mM	NDSB 195	1 mM	NDSB 195
47	D11	1.56 mM	NDSB 201	1 mM	NDSB 201
48	D12	1.56 mM	Fos-Choline-12	1 mM	Fos-Choline-12

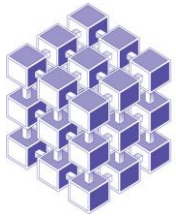




RUBIC Additive Screen Conditions E1-H12 MD1-97

Number	Position		Prefilled microplate (16 µL)		Final concentration during assay (25 µL)
49	E01	1.56 mM	CHAPS	1 mM	CHAPS
50	E02	1.56 mM	CHAPSO	1 mM	CHAPSO
51	E03	1.56 mM	OG	1 mM	OG
52	E04	1.56 mM	DM	1 mM	DM
53	E05	1.56 mM	DDM	1 mM	DDM
54	E06	39 mM	Monosaccharides mix	25 mM	Monosaccharides mix
55	E07	39 mM	D-Glucose	25 mM	D-Glucose
56	E08	39 mM	Sucrose	25 mM	Sucrose
57	E09	39 mM	Maltose	25 mM	Maltose
58	E10	78.1 mM	Carboxylic acids mix	50 mM	Carboxylic acids mix
59	E11	78.1 mM	L-Proline	50 mM	L-Proline
60	E12	78.1 mM	Glycine	50 mM	Glycine
61	F01	78.1 mM	L-Glutamic acid monosodium salt hydrate	50 mM	L-Glutamic acid monosodium salt hydrate
62	F02	781 mM	L-Glutamic acid monosodium salt hydrate	500 mM	L-Glutamic acid monosodium salt hydrate
63	F03	78.1 mM	L-Arginine	50 mM	L-Arginine
64	F04	781 mM	L-Arginine	500 mM	L-Arginine
65	F05	78.1 mM	L-Glutamic acid monosodium salt hydrate /78.1 mM L-Arginine	50 mM	L-Glutamic acid monosodium salt hydrate /50 mM L-Arginine
66	F06	781 mM	L-Glutamic acid monosodium salt hydrate /781 mM L-Arginine	500 mM	L-Glutamic acid monosodium salt hydrate /500 mM L-Arginine
67	F07	78.1 mM	Gly-Gly-Gly	50 mM	Gly-Gly-Gly
68	F08	7.81 mM	Oxaloacetic acid	5 mM	Oxaloacetic acid
69	F09	7.81 % v/v	Dimethyl sulfoxide	5 % v/v	Dimethyl sulfoxide
70	F10	7.81 % v/v	Ethylene glycol	5 % v/v	Ethylene glycol
71	F11	7.81 % v/v	Glycerol	5 % v/v	Glycerol
72	F12	31.2 % v/v	Glycerol	20 % v/v	Glycerol
73	G01	7.81 % v/v	PEG 400	5 % v/v	PEG 400
74	G02	7.81 % w/v	PEG 1000	5 % w/v	PEG 1000
75	G03	7.81 % w/v	PEG 3350	5 % w/v	PEG 3350
76	G04	7.81 mM	DTT	5 mM	DTT
77	G05	7.81 mM	TCEP	5 mM	TCEP
78	G06	7.81 mM	Biotin	5 mM	Biotin
79	G07	7.81 mM	Betaine hydrochloride	5 mM	Betaine hydrochloride
80	G08	7.81 mM	Coenzyme A	5 mM	Coenzyme A
81	G09	7.81 mM	Nicotinic acid	5 mM	Nicotinic acid
82	G10	1.56 mM	Spermidine	1 mM	Spermidine
83	G11	1.56 mM	Spermine tetrahydrochloride	1 mM	Spermine tetrahydrochloride
84	G12	1.56 mM	Sarcosine	1 mM	Sarcosine
85	H01	31.8 µM	Deoxyribonucleic acid	20 µM	Deoxyribonucleic acid
86	H02	1.56 mM	ATP/ 1.56mM Magnesium chloride	1 mM	ATP/ 1mM Magnesium chloride
87	H03	1.56 mM	ATPγS/ 1.56mM Magnesium chloride	1 mM	ATPγS/ 1mM Magnesium chloride
88	H04	1.56 mM	cAMP/ 1.56mM Magnesium chloride	1 mM	cAMP/ 1mM Magnesium chloride
89	H05	1.56 mM	GTP/ 1.56mM Magnesium chloride	1 mM	GTP/ 1mM Magnesium chloride
90	H06	1.56 mM	GTPγS/ 1.56mM Magnesium chloride	1 mM	GTPγS/ 1mM Magnesium chloride
91	H07	1.56 mM	cGMP/ 1.56mM Magnesium chloride	1 mM	cGMP/ 1mM Magnesium chloride
92	H08	1.56 mM	NADH/ 1.56mM Magnesium chloride	1 mM	NADH/ 1mM Magnesium chloride
93	H09	1.56 mM	NADPH/ 1.56mM Magnesium chloride	1 mM	NADPH/ 1mM Magnesium chloride
94	H10	7.81 mM	Polyethyleneimine 800	5 mM	Polyethyleneimine 800
95	H11	312.5 mM	Imidazole	200 mM	Imidazole
96	H12	625 mM	Imidazole	400 mM	Imidazole

*Monosaccharide and Carboxylic acid mixes are from Morpheus, MD2-100-75 and MD2-100-76 respectively. Monosaccharide Mix contains : 0.2M D-(+)-Glucose, 0.2M D-(+)-Mannose, 0.2M D-(+)-Galactose, 0.2M L-(-)-Fucose, 0.2M D-(+)-Xylose, 0.2M N-Acetyl-D-glusosamine.
Carboxylic acid mix contains: 0.2M Sodium formate, 0.2M Ammonium acetate, 0.2M Sodium citrate tribasic dihydrate, 0.2M Sodium oxamate, 0.2M Potassium sodium tartrate tetrahydrate



Molecular Dimensions

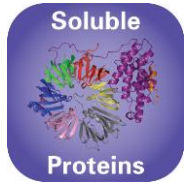


Figure 2. Layout of the of RUBIC Additive Screen

	1	2	3	4	5	6	7	8	9	10	11	12
A	water	100 mM Na Acetate	100 mM Ca Acetate	100 mM K Acetate	100 mM Ammonium Acetate	100 mM Na Sulfate	100 mM Mg Sulfate	100 mM K Sulfate	100 mM Ammonium Sulfate	100 mM Na Phosphate (monobasic)	100 mM Na Phosphate (dibasic)	100 mM K Phosphate (monobasic)
B	100 mM K Phosphate (dibasic)	100 mM Na Tartrate	100 mM Na Citrate (tribasic)	100 mM Na Malonate	100 mM Na Nitrate	100 mM Na Formate	100 mM K Formate	100 mM NaF	100 mM KF	100 mM NH4F	100 mM LiCl	100 mM NaCl
C	100 mM KCl	100 mM NH4Cl	100 mM NaI	100 mM KI	100 mM NaBr	1 mM MgCl2	1 mM CaCl2	1 mM MnCl2	1 mM NiCl2	1 mM FeCl2	1 mM ZnCl2	1 mM CoCl2
D	5 mM EDTA	5 mM EGTA	0.1 M Urea	0.5 M Urea	1 M Urea	2 M Urea	4 M Urea	150 mM Guanidine-HCl	500 mM Guanidin-HCl	1 mM NDSB-195	1 mM NDSB-201	1mM Fos Choline 12
E	1 mM CHAPS	1mM CHAPSO	1 mM OG	1mM DM	1 mM DDM	25 mM Monosaccharides mix MD2-100-75	25 mM Glucose	25 mM Sucrose	25 mM Maltose	50 mM Carboxylic acids mix MD2-100-76	50 mM Proline	50 mM Glycine
F	50 mM Glutamic acid	500 mM Glutamic acid	50 mM Arginine	500 mM Arginine	50 mM Arginine 50 mM Glutamic acid	500 mM Arginine 500 mM Glutamic acid	50mM Gly-Gly-Gly	5 mM Oxaloacetic acid	5%(v/v) DMSO	5% (v/v) Ethylene glycol	5% (v/v) Glycerol	20% (v/v) Glycerol
G	5%(v/v) PEG 400	5% (w/v) PEG 1000	5% (w/v) PEG 3350	5 mM DTT	5 mM TCEP	5 mM Biotin	5 mM Betaine	5 mM Coenzyme A	5 mM Nicotinic acid	1 mM Spermidine	1 mM Spermine	1 mM Sarcosine
H	~20 uM Deoxyribonucleic acid librairy <50 bp	1 mM ATP 1 mM MgCl2	1 mM ATPγS 1 mM MgCl2	1 mM cAMP 1 mM MgCl2	1 mM GTP 1 mM MgCl2	1 mM GTPγS 1 mM MgCl2	1 mM cGMP 1 mM MgCl2	1 mM NADH 1 mM MgCl2	1 mM NADPH 1 mM MgCl2	5 mM Polyethylenimine	200 mM Imidazole	400 mM Imidazole

Salts
Monovalent ions
Multivalent ions, chelating reageants
Chaotropic reagents
Non detergeant , detergents,
Carbohydrates
Carboxylic acids, amino acids (racemic)
Reducing reagents
Polyols
Co-factor, polyamines
Nucleotides
Imidazole

Concentrations shown above are final concentrations based on 25 µl assay
(16 µL RUBIC Additive Screen + 5 µL 5X Buffer* + 2 µL Protein sample + 2 µL SYPRO Orange dye diluted stock solution).
*5X Buffer can be the buffers provided or your own stock.

Abbreviations:
PEG: Poly Ethylene Glycol, **EDTA:** Ethylenediaminetetraacetic acid, **CHAPS**: 3-[(3-Cholamidopropyl)-Dimethylammonio]-1-Propane Sulfonate/N,N-Dimethyl-3-Sulfo-N-[3-[[3α,5β,7α,12α)-3,7,12-Trihydroxy-24-Oxocholan-24-yl]Amino]propyl]-1-Propanaminium Hydroxide, Inner Salt, **CHAPSO:** 3-[(3-Cholamidopropyl)dimethylammonio]-2-Hydroxy-1-Propanesulfonate, **OG:** n-Octyl-β-D-Glycopyranoside. **DM:** n-Decyl-β-D-maltopyranoside, **DDM**: n-Dodecyl-β-D-Maltopyranoside, **DTT:** DL-Dithiothreitol; **TCEP:** Tris(2-carboxyethyl)phosphine hydrochloride, **ATP:** Adenosine 5'triphosphate disodium salt hydrate, **ATPγS:** Adenosine 5'-[γ-thio]triphosphate tetralithium salt, **cAMP:** Adenosine 3',5'-cyclic monophosphate sodium salt monohydrate, **GTP:** Guanosine 5'-triphosphate sodium salt hydrate, **GTPγS:** Guanosine 5'-[γ-thio]triphosphate tetralithium salt, **cGMP:** Guanosine 3',5'-cyclic monophosphate sodium salt, **NADH:** β-Nicotinamide adenine dinucleotide, reduced dipotassium salt; **NADPH:** β-Nicotinamide adenine dinucleotide phosphate, reduced tetra(cyclohexylammonium) salt, **HEPES:** 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid), **MES:** 2-(N-Morpholino)ethanesulfonic acid, **Bis-Tris:** 2,2-Bis(hydroxymethyl)-2,2',2"-nitrilotriethanol, **MOPS:** 3-(N-Morpholino)propanesulfonic acid, **Tris-HCl:** Trizma® hydrochloride, **BICINE:** 2-(Bis(2-hydroxyethyl)amino)acetic acid, **CHES**: 2-(Cyclohexylamino)ethanesulfonic acid

Manufacturer's safety data sheets are available from our website.

Re-Ordering details:

Catalogue Description	Pack size	Catalogue Code
RUBIC Buffer Screen	96 x 0.5 mL	MD1-96
RUBIC Additive Screen	96 x 0.25 mL (+ 24 x 1.5 mL buffers)	MD1-97
RUBIC Buffer Set*	48 x 11 mL	MD1-96-BUFFER

*can be used in synergy with the additive screen or to set-up a customized TF experiment. Buffers are A1 to B24 at 0.5M Buffer, C1 to D24 at 0.5M Buffer+ 1.25M NaCl from the RUBIC Buffer Screen.

Single Reagents

RUBIC Buffer Screen single reagents	10 mL	MDSR-96-well number
RUBIC Additive Screen single reagents	100 µL	MDSR-97-well number

Molecular Dimensions

Soluble Proteins

The Durham pH Screen MD1-101

A pre-crystallization ThermoFluor® screen for finding optimal protein crystallization conditions.

Simplifies the discovery of protein-specific stabilising conditions and helps generate a starting-point for fine grid screening.

MD1-101 is presented as 96 x 0.5 mL conditions in a deep-well block.

Features of The Durham pH Screen:

- Broad range of pH from 4 – 11.
- 28 different buffer molecules.
- Discover conditions that significantly (de)stabilise your protein.
- Improve protein purification and characterisation.
- Determine optimal crystallization conditions.
- Ideal for screening fragment ligands in Fragment-Based Drug Design (FBDD).
- Use alongside The Durham Salt Screen and RUBIC Screens.
- Use with NAMI* a GUI-based python program to get rapid high-throughput data analysis of your results.

Introduction

The Durham pH Screen allows the rapid characterization of the effects of both buffer identity and pH on protein behaviour. It can be used in assays to determine such properties as solubility, isothermal stability, and thermal denaturation point. Thermal denaturation data is not typically needed on a specific protein (unless it is used in bioindustrial processes), but buffer molecules that influence the thermal denaturation point usually also influence properties such as protease resistance, crystallizability, isothermal stability, and solubility.

Most researchers are interested in changes in thermal denaturation point and the assays to determine this are collectively named thermal shift assays (TSA). Thermal shift data can be obtained quickly through a wealth of techniques, with and without specialized machines. The most widely used is the ThermoFluor assay (also known as differential scanning fluorimetry) which does not require any specialized machines.

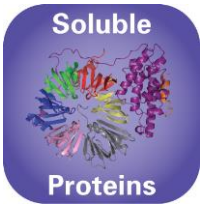
The Durham pH Screen covers a broad pH range, from 4 – 11, using 28 different buffer molecules, including the buffer molecules that most frequently occur in the PDB. As the pH values of different buffers overlap in this screen, it is easier to deconvolute the effects of pH and type of buffer on protein stability.

Screening for optimal protein crystallization conditions.

It is best to sample as broad a range of potential crystallization reagents as possible. Including **The Durham pH** and **Salt Screens** in your initial ThermoFluor experiments will facilitate the discovery of protein-specific stabilising conditions and generate a starting-point for fine-grid screening.

Combining both the Durham screens with the rapid high-throughput data analysis offered by the GUI-based python program, NAMI (Grøftehauge *et al.*, 2015), creates a powerful tool for pin-pointing desirable conditions for the crystallization of your protein.

*NAMI is a free-of-charge program. See Page 5 for further details.



Why use ThermoFluor Screens?

Protein Purification: pH should be chosen to be as close to the protein stability optimum as practically possible but many chromatographic methods have distinct pH requirements, typically around neutral. So it is important to choose a buffering molecule that provides the greatest stability. Greater protein stability leads to less proteolysis and less aggregation and therefore greater purification yields.

Crystallography: Ligands may also induce a conformational change that decreases the thermal denaturation point rather than increases it; this may be beneficial or deleterious towards crystallization. It is somewhat common for protein to be crystallized in complex with a pH buffer molecule as they are often present in excess in the crystallization drop. A buffer molecule can work as a ligand, and ligands mostly increase the propensity towards crystallizing and diffracting; therefore it is useful to obtain information about their influence on the specific protein of interest.

A pH buffer molecule may also be non-specifically destabilising in the same manner as salt or urea and this is generally considered detrimental to crystallization. Even with a protein crystal structure, it can be very hard to predict the pH at which a crystal forms. However, if the protein is denatured at a specific pH it would seem very unlikely that a crystal would form at that pH.

Fragment-Based Drug Design (FBDD): Fragments are very small organic molecules and their potential binding affinity as ligands are thus limited. When screening for fragment ligands you should use a pH buffer that does not interact specifically with the protein target; stabilising pH buffer molecules may bind in the same pocket as the fragments.

*NAMI is a free-of-charge program. See Page 5 for further details.

Durham Screens (pH and Salt screens)
Thermal Shift Assay Protocol (See Figure 1)

Starting Materials:

- Durham pH or Salt screen in a 96 deep-well block
- 4 µl of 5,000 X SYPRO Orange in DMSO
- 1 ml of pure 0.5-2.0 mg/ml protein sample
- One 96-well PCR plate, specific to the RT-PCR machine being used
- One self-adhesive plate seal, suitable for RT-PCR

Protocol (summarised in Figure 1):

1. Transfer 10 µl of each screen condition into the corresponding well of the PCR plate. This can be done manually using an automatic pipette or using a liquid handling robot if available.

2. Combine 4 µl of 5,000 X SYPRO Orange in DMSO with 1 ml of pure protein sample. Add 10 µl of this solution (protein and SYPRO Orange) into each well of a PCR plate.

3. **The final components of each well will be 1 X screen solution, 10 X SYPRO Orange, and half the original protein concentration.**

4. Seal the plate with a self-adhesive seal suitable for RT-PCR. Then centrifuge the plate at 1,000 RPM for 2 minutes. The assay is now ready to be run.

5. Many commonly used RT-PCR machines can be adapted to run a thermal shift assay. The recommended programme for TSA data collection is:
   - sample a temperature gradient of 24-96˚C,
   - increasing 1˚C per minute,
   - with a fluorescence reading being taken in every well at every temperature increment,
   - using a 455-485 nm wavelength range for excitation,
   - and emission collected between 567-596 nm.

Please note the exact wavelengths will depend on the filters present in your RT-PCR machine. Simply ensure the excitation and emission maxima of SYPRO Orange at 470 nm and 569 nm, respectively, are covered.

6. On completion of the assay, appropriately dispose of the plate. Export the data as a .csv file (*comma separated values*) for analysis in NAMI (or other suitable software).

*NAMI is a free-of-charge program. See Page 5 for further details.

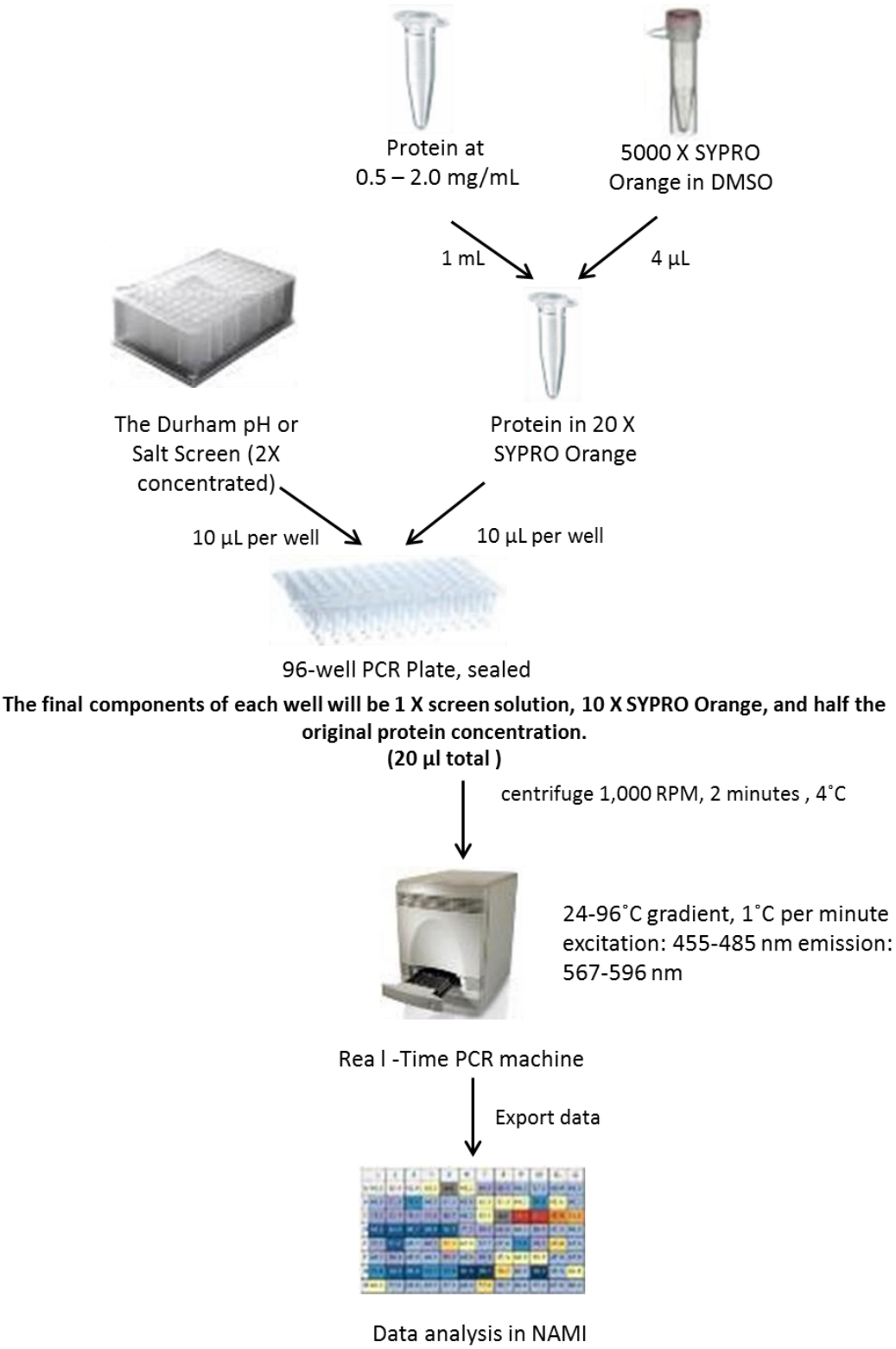


Figure 1. An overview of the thermal shift assay protocol

*NAMI is a free-of-charge program. See Page 5 for further details.

NAMI is available free of charge by following the download instructions at https://www.dur.ac.uk/chemistry/academic-groups/ehmke.pohl/nami/downloads/- also see the Data Analysis in NAMI user guide.

Please cite the following paper when using the program:

GrØftehauge MK, Hajizadeh, NR, Swann MR, Pohl E. Protein-ligand interactions investigated by thermal shift assays (TSA) and dual polarization interferometry (DPI). (2015) Acta Cryst. D71:36.44

Formulation Notes:

The Durham pH and Salt Screen reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 μm filters. No preservatives are added. Prepared at room temperature.
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.
Contact and product details can be found at www.moleculardimensions.com
Enquiries regarding Durham pH and Salt Screen formulation, interpretation of results, or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

Storage: Screens should be kept at 4˚C, but they can be at room temperature for screen set-up.

References

GrØftehauge MK, Hajizadeh, NR, Swann MR, Pohl E. Protein-ligand interactions investigated by thermal shift assays (TSA) and dual polarization interferometry (DPI). (2015) Acta Cryst. D71:36.44.

*NAMI is a free-of-charge program. See Page 5 for further details.

Molecular Dimensions

Soluble Proteins

The Durham pH Screen Conditions A1-H12* MD1-101

Well #	Conc	Reagent	Well #	Conc	Reagent
A1	100 %	Ultrapure water	E1	0.2 M	Sodium phosphate pH 6.3
A2	100 %	Ultrapure water	E2	0.2 M	Sodium phosphate pH 6.8
A3	8 M	Urea	E3	0.2 M	Sodium phosphate pH 7.3
A4	0.2 M	Citrate pH 4.1	E4	0.2 M	PIPES pH 6.3
A5	0.2 M	Citrate pH 4.6	E5	0.2 M	PIPES pH 6.8
A6	0.2 M	Citrate pH 5.1	E6	0.2 M	PIPES pH 7.3
A7	0.2 M	Acetic acid pH 4.2	E7	0.2 M	Imidazole pH 6.6
A8	0.2 M	Acetic acid pH 4.7	E8	0.2 M	Imidazole pH 7.1
A9	0.2 M	Acetic acid pH 5.2	E9	0.2 M	Imidazole pH 7.6
A10	0.2 M	Succinic acid pH 4.4	E10	0.2 M	MOPS pH 6.6
A11	0.2 M	Succinic acid pH 4.9	E11	0.2 M	MOPS pH 7.1
A12	0.2 M	Succinic acid pH 5.4	E12	0.2 M	MOPS pH 7.6
B1	0.2 M	DL-Malic acid pH 4.3	F1	0.2 M	Bis-Tris propane pH 6.6
B2	0.2 M	DL-Malic acid pH 4.8	F2	0.2 M	Bis-Tris propane pH 7.1
B3	0.2 M	DL-Malic acid pH 5.3	F3	0.2 M	Bis-Tris propane pH 7.6
B4	0.2 M	L-Tartaric acid pH 4.3	F4	0.2 M	HEPES pH 7.0
B5	0.2 M	L-Tartaric acid pH 4.8	F5	0.2 M	HEPES pH 7.5
B6	0.2 M	L-Tartaric acid pH 5.3	F6	0.2 M	HEPES pH 8.0
B7	0.2 M	Propionic acid pH 4.3	F7	0.2 M	Tricine pH 7.5
B8	0.2 M	Propionic acid pH 4.8	F8	0.2 M	Tricine pH 8.0
B9	0.2 M	Propionic acid pH 5.3	F9	0.2 M	Tricine pH 8.5
B10	0.2 M	Malonic acid pH 5.2	F10	0.2 M	EPPS pH 7.5
B11	0.2 M	Malonic acid pH 5.7	F11	0.2 M	EPPS pH 8.0
B12	0.2 M	Malonic acid pH 6.2	F12	0.2 M	EPPS pH 8.5
C1	0.2 M	Citrate pH 5.5	G1	0.2 M	Tris pH 7.7
C2	0.2 M	Citrate pH 6.0	G2	0.2 M	Tris pH 8.2
C3	0.2 M	Citrate pH 6.5	G3	0.2 M	Tris pH 8.7
C4	0.2 M	Succinic acid pH 5.6	G4	0.2 M	BICINE pH 7.7
C5	0.2 M	Succinic acid pH 6.1	G5	0.2 M	BICINE pH 8.2
C6	0.2 M	Succinic acid pH 6.6	G6	0.2 M	BICINE pH 8.7
C7	0.2 M	MES pH 5.6	G7	0.2 M	TAPS pH 7.9
C8	0.2 M	MES pH 6.1	G8	0.2 M	TAPS pH 8.4
C9	0.2 M	MES pH 6.6	G9	0.2 M	TAPS pH 8.9
C10	0.2 M	Maleic acid pH 5.7	G10	0.2 M	Bis-Tris propane pH 8.5
C11	0.2 M	Maleic acid pH 6.2	G11	0.2 M	Bis-Tris propane pH 9.0
C12	0.2 M	Maleic acid pH 6.7	G12	0.2 M	Bis-Tris propane pH 9.5
D1	0.2 M	Sodium cacodylate pH 5.7	H1	0.2 M	Boric acid pH 8.6
D2	0.2 M	Sodium cacodylate pH 6.2	H2	0.2 M	Boric acid pH 9.1
D3	0.2 M	Sodium cacodylate pH 6.7	H3	0.2 M	Boric acid pH 9.6
D4	0.2 M	ADA pH 6.1	H4	0.2 M	CHES pH 8.8
D5	0.2 M	ADA pH 6.6	H5	0.2 M	CHES pH 9.3
D6	0.2 M	ADA pH 7.1	H6	0.2 M	CHES pH 9.8
D7	0.2 M	Bis-Tris pH 6.1	H7	0.2 M	Glycine pH 9.2
D8	0.2 M	Bis-Tris pH 6.6	H8	0.2 M	Glycine pH 9.7
D9	0.2 M	Bis-Tris pH 7.1	H9	0.2 M	Glycine pH 10.2
D10	0.2 M	ACES pH 6.3	H10	0.2 M	CAPS pH 9.9
D11	0.2 M	ACES pH 6.8	H11	0.2 M	CAPS pH 10.4
D12	0.2 M	ACES pH 7.3	H12	0.2 M	CAPS pH 10.9

*concentrations shown are not final concentrations. For final concentrations- see Figure 2.

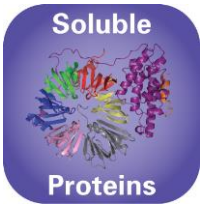
*NAMI is a free-of-charge program. See Page 5 for further details.

Figure 2:- Layout of the of Durham pH Screen

	1	2	3	4	5	6	7	8	9	10	11	12
A	water	water	4 M urea	100 mM citric acid pH 4.1	100 mM citric acid pH 4.6	100 mM citric acid pH 5.1	100 mM acetic acid pH 4.2	100 mM acetic acid pH 4.7	100 mM acetic acid pH 5.2	100 mM succinic acid pH 4.4	100 mM succinic acid pH 4.9	100 mM succinic acid pH 5.4
B	100 mM malic acid pH 4.3	100 mM malic acid pH 4.8	100 mM malic acid pH 5.3	100 mM tartaric acid pH 4.3	100 mM tartaric acid pH 4.8	100 mM tartaric acid pH 5.3	100 mM propionic acid pH 4.3	100 mM propionic acid pH 4.8	100 mM propionic acid pH 5.3	100 mM malonic acid pH 5.2	100 mM malonic acid pH 5.7	100 mM malonic acid pH 6.2
C	100 mM citric acid pH 5.5	100 mM citric acid pH 6.0	100 mM citric acid pH 6.5	100 mM succinic acid pH 5.6	100 mM succinic acid pH 6.1	100 mM succinic acid pH 6.6	100 mM MES pH 5.6	100 mM MES pH 6.1	100 mM MES pH 6.6	100 mM maleic acid pH 5.7	100 mM maleic acid pH 6.2	100 mM maleic acid pH 6.7
D	100 mM sodium cacodylate pH 5.7	100 mM sodium cacodylate pH 6.2	100 mM sodium cacodylate pH 6.7	100 mM ADA pH 6.1	100 mM ADA pH 6.6	100 mM ADA pH 7.1	100 mM bisTRIS pH 6.1	100 mM bisTRIS pH 6.6	100 mM bisTRIS pH 7.1	100 mM ACES pH 6.3	100 mM ACES pH 6.8	100 mM ACES pH 7.3
E	100 mM phosphate pH 6.3	100 mM phosphate pH 6.8	100 mM phosphate pH 7.3	100 mM PIPES pH 6.3	100 mM PIPES pH 6.8	100 mM PIPES pH 7.3	100 mM imidazole pH 6.6	100 mM imidazole pH 7.1	100 mM imidazole pH 7.6	100 mM MOPS pH 6.6	100 mM MOPS pH 7.1	100 mM MOPS pH 7.6
F	100 mM bisTRIS propane pH 6.6	100 mM bisTRIS propane pH 7.1	100 mM bisTRIS propane pH 7.6	100 mM HEPES pH 7.0	100 mM HEPES pH 7.5	100 mM HEPES pH 8.0	100 mM tricine pH 7.5	100 mM tricine pH 8.0	100 mM tricine pH 8.5	100 mM EPPS pH 7.5	100 mM EPPS pH 8.0	100 mM EPPS pH 8.5
G	100 mM TRIS pH 7.7	100 mM TRIS pH 8.2	100 mM TRIS pH 8.7	100 mM bicine pH 7.7	100 mM bicine pH 8.2	100 mM bicine pH 8.7	100 mM TAPS pH 7.9	100 mM TAPS pH 8.4	100 mM TAPS pH 8.9	100 mM bisTRIS propane pH 8.5	100 mM bisTRIS propane pH 9.0	100 mM bisTRIS propane pH 9.5
H	100 mM boric acid pH 8.6	100 mM boric acid pH 9.1	100 mM boric acid pH 9.6	100 mM CHES pH 8.8	100 mM CHES pH 9.3	100 mM CHES pH 9.8	100 mM glycine pH 9.2	100 mM glycine pH 9.7	100 mM glycine pH 10.2	100 mM CAPS pH 9.9	100 mM CAPS pH 10.4	100 mM CAPS pH 10.9

Concentrations shown are final concentrations. The final components of each well will be 1 X screen solution, 10 X SYPRO Orange, and half the original protein concentration.

*NAMI is a free-of-charge program. See Page 5 for further details.



Abbreviations:
MES; 2-(N-morpholino)ethanesulfonic acid, **ADA**; N-(2-Acetamido)iminodiacetic acid**, PIPES**; 1,4-Piperazinediethanesulfonic acid, **MOPS**; 3-(N-Morpholino)propanesulfonic acid **TAPS**; N-[Tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid **Tris**; Trizma base **CHES**; 2-(Cyclohexylamino)ethanesulfonic acid, **CAPS**; 3-(Cyclohexylamino)-1-propanesulfonic acid, **EPPS**; 4-(2-Hydroxyethyl)-1-piperazinepropanesulfonic acid, **HEPES**; 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid), **ACES**: N-(2-Acetamido)-2-aminoethanesulfonic acid, N-(Carbamoylmethyl)taurine, **BICINE**: 2-(Bis(2-hydroxyethyl)amino)acetic acid

Manufacturer's safety data sheets are available from our website.

Re-Ordering Details:

Catalogue Description	Pack size	Catalogue Code
The Durham pH Screen	96 x 0.5 mL	MD1-101
The Durham Salt Screen	96 x 0.5 mL	MD1-102
RUBIC Buffer Screen	96 x 0.5 mL	MD1-96
RUBIC Additive Screen	96 x 0.25 mL	MD1-97
RUBIC Buffer Set*	48 x 11 mL	MD1-96-BUFFER
Single Reagents		
The Durham pH Screen single reagents	96 x 0.5 mL	MDSR-101-well number
The Durham Salt Screen	96 x 0.5 mL	MDSR-102-well number
RUBIC Buffer Screen single reagents	10 mL	MDSR-96-well number
RUBIC Additive Screen single reagents	various volumes	See website for more details.

All stocks are available to buy from Molecular Dimensions.

*The RUBIC Buffer Set contains buffers A1 to B24 at 0.5M Buffer, C1 to D24 at 0.5M Buffer+ 1.25M NaCl from the RUBIC Buffer Screen.

*NAMI is a free-of-charge program. See Page 5 for further details.

Molecular Dimensions

Soluble Proteins

The Durham Salt Screen MD1-102

A pre-crystallization ThermoFluor® salt screens for finding optimal protein crystallization conditions.

Simplifies the discovery of protein-specific stabilising conditions and helps generate a starting-point for protein crystallization.

MD1-102 is presented as 96 x 0.5 mL conditions in a deep-well block.

Features of The Durham Salt Screens:

- Broad range (>30) of salts including chaotropic reagents, monovalent and multivalent ions, chelating agents, heavy metal salts and reducing reagents.
- Discover conditions that significantly (de)stabilise your protein.
- Improve protein purification and characterisation.
- Determine optimal crystallization conditions.
- Use alongside The Durham pH Screen and RUBIC Screens.
- Use with NAMI* a GUI-based python program to get rapid high-throughput data analysis of your results.

Introduction

The Durham Salt Screen is a pre-crystallization ThermoFluor screen designed to be used in tandem with **The Durham pH Screen**. It consists of more than 30 different common salts, including chemotropic reagents, monovalent and multivalent ions, chelating agents, heavy metal salts and reducing reagents. It allows the rapid characterization of the effects of salts and concentrations on protein behaviour. It can be used in assays to determine such properties as solubility, isothermal stability, and thermal denaturation point.

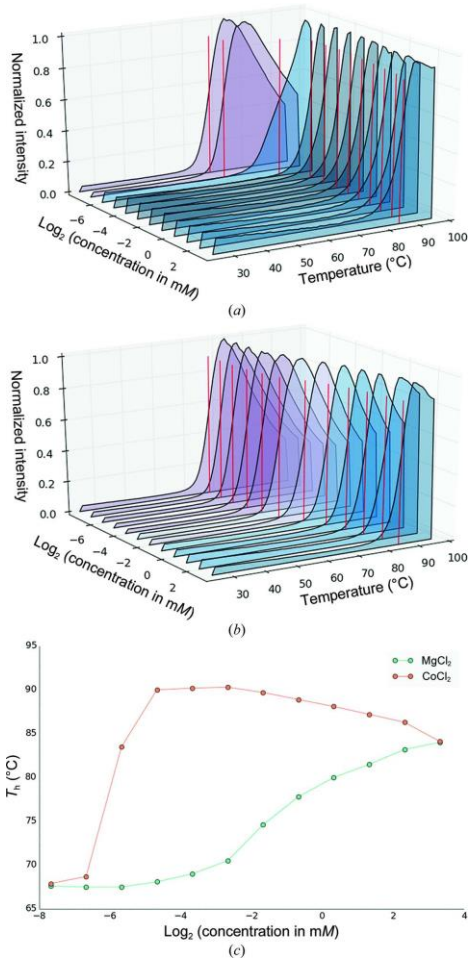
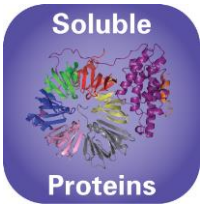


Figure 1: Examples of the analysis part of *NAMI*. (*a*) Waterfall plot of a follow-up screen in which the effect of the serial dilution of divalent metals on glucose isomerase is shown. The starting concentration of CoCl2 is10 mM. Purple curves indicate no significant difference from the reference; blue curves indicate a significant shift towards higher Th. (b)Waterfall plot of increasing MgCl2 concentration starting at 10 mM. (c)Melting temperature Th as a function of the concentration of CoCl2 andMgCl2, respectively. ***Grøftehauge et al. Volume 71 | Part 1 | January 2015 | Pages 36–44 | 10.1107/S1399004714016617***

*NAMI is a free-of-charge program. See Page 5 for further details.



Why use ThermoFluor Screens?

Researchers are interested in changes in the thermal denaturation point and the assays to determine this are collectively named thermal shift assays (TSA). Thermal shift data can be obtained quickly through a wealth of techniques, with and without specialized machines. The most widely used is the ThermoFluor assay (also known as differential scanning fluorimetry, DSF or thermal shift assay, TSA) which does not require any specialized machines. ThermoFluor screens are a great way of finding out what conditions are the best suited for your protein. In other words you can find out what destabilizes your protein or stabilizes it.

Protein Purification: The type of salt and concentration should be chosen to be as close to the protein stability optimum as practically possible but many chromatographic methods have distinct salt requirements, and typically NaCl is the go-to choice of salt but not necessarily the best one for your protein. So it is important to choose a salt molecule that provides the greatest stability. Greater protein stability leads to less proteolysis and less aggregation and therefore greater purification yields.

Crystallography: Ligands may also induce a conformational change that decreases the thermal denaturation point rather than increases it; this may be beneficial or detrimental towards crystallization. It is somewhat common for protein to be crystallized in complex with a salt (ion) molecule as they are often present in excess in the crystallization drop. Salts are both common precipitating agents and additives in crystallization conditions; therefore it is useful to obtain information about the influence of specific salts on the specific protein of interest.

Screening for optimal protein crystallization conditions.

It is best to sample as broad a range of potential crystallization reagents as possible. Including **The Durham pH** and **Salt Screens** in your initial ThermoFluor experiments will facilitate the discovery of protein-specific stabilising conditions and generate a starting-point for fine-grid screening. Combining both the Durham screens with the rapid high-throughput data analysis offered by the GUI-based python program, NAMI (GrØftehauge *et al*., 2015), creates a powerful tool for pin-pointing desirable conditions for the crystallization of your protein.

*NAMI is a free-of-charge program. See Page 5 for further details.

Durham Screens (pH and Salt screens)
Thermal Shift Assay Protocol (See Figure 2)

Starting Materials:

- Durham pH or Salt screen in a 96 deep-well block
- 4 µl of 5,000 X SYPRO Orange in DMSO
- 1 ml of pure protein sample typically at 0.5-2.0 mg/ml
- One 96-well PCR plate, specific to the RT-PCR machine being used
- One self-adhesive plate seal, suitable for RT-PCR

Protocol (summarised in Figure 1):

1. Transfer 10 µl of each screen condition into the corresponding well of the PCR plate. This can be done manually using an automatic pipette or using a liquid handling robot if available.

2. Combine 4 µl of 5,000 X SYPRO Orange in DMSO with 1 ml of protein sample. Add 10 µl of this solution (protein and SYPRO Orange) into each well of a PCR plate.

3. **The final components of each well will be 1 X screen solution, 10 X SYPRO Orange, and half the original protein concentration.**

4. Seal the plate with a self-adhesive seal suitable for RT-PCR. Then centrifuge the plate at 1,000 RPM for 2 minutes. The assay is now ready to be run.

5. Many commonly used RT-PCR machines can be adapted to run a thermal shift assay. The recommended programme for TSA data collection is:
   - sample a temperature gradient of 24-96˚C,
   - increasing 1˚C per minute,
   - with a fluorescence reading being taken in every well at every temperature increment,
   - using a 455-485 nm wavelength range for excitation,
   - and emission collected between 567-596 nm.

Please note the exact wavelengths will depend on the filters present in your RT-PCR machine. Simply ensure the excitation and emission maxima of SYPRO Orange at 470 nm and 569 nm, respectively, are covered.

6. On completion of the assay, appropriately dispose of the plate. Export the data as a .csv (*comma separated values*) file for analysis in NAMI (or other suitable software).

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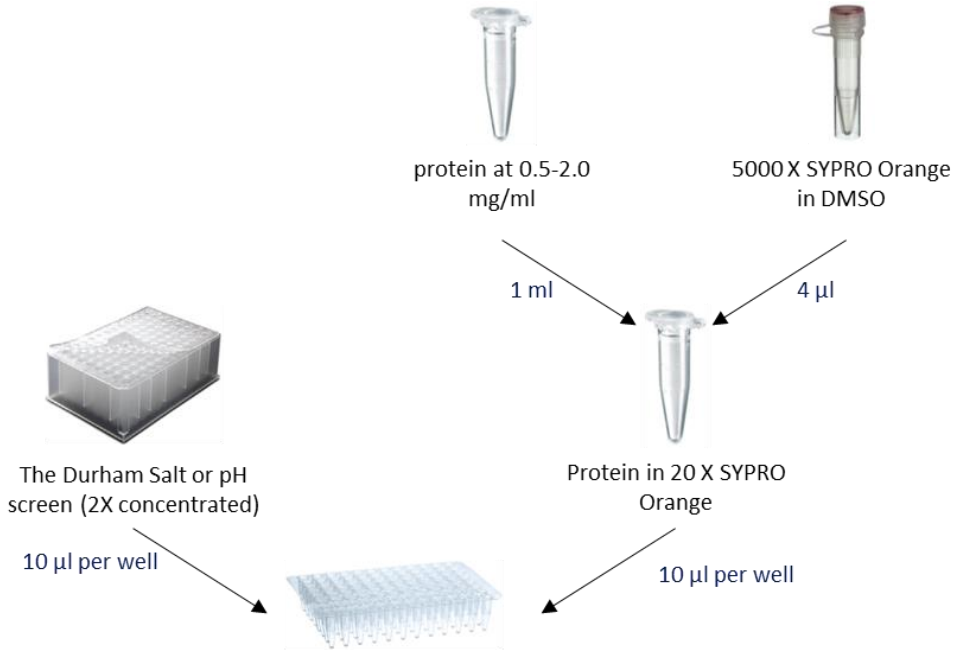

96-well PCR plate (or semi-skirted PCR plate for FAST systems) sealed.

The final components of each well will be 1 X Durham screen + 10 X SYPRO Orange + half original protein concentration. (20 µl total)

centrifuge 1,000 RPM, 2 minutes, 4˚C

Real-Time PCR Machine

24-96˚C gradient, 1˚C per minute
excitation: 455-485 nm
emission: 567-596 nm or 505-535 nm

export data

	1	2	3	4	5	6	7	8	9	10	11	12
A	65.9	65.9	65.9	63.7	N/S	63.2	68.7	68.5	68.0	67.2	66.9	66.5
B	66.8	71.3	73.4	66.6	67.2	66.6	62.3	63.3	64.2	76.5	65.8	66.0
C	72.2	72.7	71.5	70.6	69.7	68.0	63.4	N/S	27.0	31.0	41.9	51.5
D	84.8	84.8	84.7	84.7	84.7	70.5	69.3	69.0	68.2	69.3	67.5	68.2
E	70.9	82.6	67.7	68.3	62.1	65.8	67.3	67.6	73.1	68.9	54.6	67.4
F	68.0	68.5	68.0	66.4	70.2	67.6	68.8	65.5	65.6	65.7	67.9	67.9
G	76.4	84.1	82.8	76.0	75.9	86.8	88.0	56.2	66.7	89.7	66.5	65.1
H	65.7	67.6	67.6	67.5	67.5	67.9	57.2	66.7	66.2	67.1	68.0	66.9

data analysis in NAMI

Figure 2. An overview of the thermal shift assay protocol

*NAMI is a free-of-charge program. See Page 5 for further details.

NAMI is available free of charge by following the download instructions at https://www.dur.ac.uk/chemistry/academic-groups/ehmke.pohl/nami/downloads/- also see the Data Analysis in NAMI user guide.

Please cite the following paper when using the program:

GrØftehauge MK, Hajizadeh, NR, Swann MR, Pohl E. Protein-ligand interactions investigated by thermal shift assays (TSA) and dual polarization interferometry (DPI). (2015) Acta Cryst. D71:36.44

Formulation Notes:

Durham pH and Salt Screen reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added. Prepared at room temperature.
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.

Contact and product details can be found at www.moleculardimensions.com
Enquiries regarding Durham pH and Salt Screen formulation, interpretation of results, or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

Storage: Screens should be kept at 4˚C, but they can be at room temperature for screen set-up.

References

GrØftehauge MK, Hajizadeh, NR, Swann MR, Pohl E. Protein-ligand interactions investigated by thermal shift assays (TSA) and dual polarization interferometry (DPI). (2015) Acta Cryst. D71:36.44.

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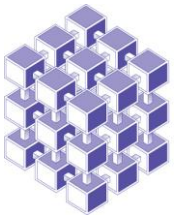
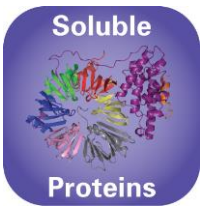




The Durham Salt Screen Conditions A1-H12* MD1-102

Well #	Conc	Reagent	Well #	Conc	Reagent
A1		Ultrapure water	E1	1 M	Lithium chloride
A2		Ultrapure water	E2	0.4 M	Lithium chloride
A3	8 M	Urea	E3	1 M	Rubidium chloride
A4	6 M	Guanidine hydrochloride	E4	0.4 M	Rubidium chloride
A5	2 M	Guanidine hydrochloride	E5	1 M	Cesium chloride
A6	1.6 M	Guanidine hydrochloride	E6	0.4 M	Cesium chloride
A7	1.2 M	Guanidine hydrochloride	E7	0.8 M	Sodium fluoride
A8	0.8 M	Guanidine hydrochloride	E8	0.2 M	Sodium fluoride
A9	0.4 M	Guanidine hydrochloride	E9	3 M	Sodium bromide
A10	10 M	Guanidine hydrochloride	E10	0.8 M	Sodium bromide
A11	1 M	Sodium citrate tribasic dihydrate	E11	0.2 M	Sodium bromide
A12	0.4 M	Sodium citrate tribasic dihydrate	E12	0.8 M	Sodium iodide
B1	3 M	Sodium malonate dibasic monohydrate	F1	0.2 M	Sodium iodide
B2	2 M	Sodium malonate dibasic monohydrate	F2	0.8 M	Magnesium chloride hexahydrate
B3	1.6 M	Sodium malonate dibasic monohydrate	F3	0.01 M	Magnesium chloride hexahydrate
B4	1.2 M	Sodium malonate dibasic monohydrate	F4	0.01 M	Calcium chloride dihydrate
B5	0.8 M	Sodium malonate dibasic monohydrate	F5	0.01 M	Strontium chloride hexahydrate
B6	0.4 M	Sodium malonate dibasic monohydrate	F6	0.002 M	Zinc chloride
B7	3 M	Ammonium sulfate	F7	0.0002 M	Zinc chloride
B8	2 M	Ammonium sulfate	F8	0.002 M	Nickel(II) chloride hexahydrate
B9	1.6 M	Ammonium sulfate	F9	0.0002 M	Nickel(II) chloride hexahydrate
B10	1.2 M	Ammonium sulfate	F10	0.01 M	Manganese(II) chloride hexahydrate
B11	0.8 M	Ammonium sulfate	F11	0.001 M	Manganese(II) chloride hexahydrate
B12	0.4 M	Ammonium sulfate	F12	0.002 M	Cobalt(II) chloride hexahydrate
C1	3 M	Sodium chloride	G1	0.0002 M	Cobalt(II) chloride hexahydrate
C2	2 M	Sodium chloride	G2	0.002 M	Copper(II) sulfate
C3	1.6 M	Sodium chloride	G3	0.0002 M	Copper(II) sulfate
C4	1.2 M	Sodium chloride	G4	0.002 M	Cadmium sulfate 8/3-hydrate
C5	0.8 M	Sodium chloride	G5	0.01 M	EDTA pH 7.5
C6	0.4 M	Sodium chloride	G6	0.01 M	EGTA pH 7.5
C7	3 M	Ammonium chloride	G7	0.004 M	I3C
C8	2 M	Ammonium chloride	G8	0.004 M	Lanthanum(III) nitrate hexahydrate
C9	1.6 M	Ammonium chloride	G9	0.004 M	Praseodymium(III) chloride hydrate
C10	1.2 M	Ammonium chloride	G10	0.004 M	Neodymium(III) chloride hexaydrate
C11	0.8 M	Ammonium chloride	G11	0.004 M	Samarium(III) chloride hexahydrate
C12	0.4 M	Ammonium chloride	G12	0.004 M	Europium(III) chloride hexahydrate
D1	2 M	Magnesium sulfate heptahydrate	H1	0.004 M	Gadolinium(III) chloride hexahydrate
D2	1.6 M	Magnesium sulfate heptahydrate	H2	0.004 M	Dysprosium(III) chloride hexahydrate
D3	1.2 M	Magnesium sulfate heptahydrate	H3	0.004 M	Holmium(III) chloride hexahydrate
D4	0.8 M	Magnesium sulfate heptahydrate	H4	0.004 M	Ytterbium(III) chloride hexahydrate
D5	0.4 M	Magnesium sulfate heptahydrate	H5	0.004 M	Lutetium(III) chloride hexahydrate
D6	2 M	Sodium sulfate	H6	0.01 M	Sodium phosphate dibasic
D7	1.6 M	Sodium sulfate	H7	0.01 M	Sodium orthovanadate
D8	1.2 M	Sodium sulfate	H8	0.01 M	Sodium tungstate
D9	0.8 M	Sodium sulfate	H9	0.01 M	Sodium molybdate
D10	0.4 M	Sodium sulfate	H10	0.01 M	DTT
D11	1 M	Potassium chloride	H11	0.01 M	TCEP pH 7.0
D12	0.4 M	Potassium chloride	H12	0.01 M	β-Mercaptoethanol (β-ME)

*concentrations shown are not final concentrations. For final concentrations- see Figure 3.

*NAMI is a free-of-charge program. See Page 5 for further details.

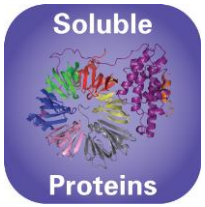
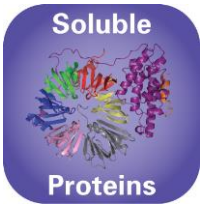


Figure 3:- Layout of the of Durham Salt Screen

	1	2	3	4	5	6	7	8	9	10	11	12
A	water	water	4 M urea	3.0 M Gu-HCl	1.0 M Gu-HCl	0.8 M Gu-HCl	0.6 M Gu-HCl	0.4 M Gu-HCl	0.2 M Gu-HCl	5 mM Gu-HCl	0.5 M Na_3 citrate	0.2 M Na_3 citrate
B	1.5 M Na_2 malonate	1.0 M Na_2 malonate	0.8 M Na_2 malonate	0.6 M Na_2 malonate	0.4 M Na_2 malonate	0.2 M Na_2 malonate	1.5 M $(NH_4)_2SO_4$	1.0 M $(NH4)_2SO_4$	0.8 M $(NH_4)_2SO_4$	0.6 M $(NH_4)_2SO_4$	0.4 M $(NH_4)_2SO_4$	0.2 M $(NH_4)_2SO_4$
C	1.5 M NaCl	1.0 M NaCl	0.8 M NaCl	0.6 M NaCl	0.4 M NaCl	0.2 M NaCl	1.5 M NH_4Cl	1.0 M NH_4Cl	0.8 M NH_4Cl	0.6 M NH_4Cl	0.4 M NH_4Cl	0.2 M NH_4Cl
D	1.0 M $MgSO_4$	0.8 M $MgSO_4$	0.6 M $MgSO_4$	0.4 M $MgSO_4$	0.2 M $MgSO_4$	1.0 M Na_2SO_4	0.8 M Na_2SO_4	0.6 M Na_2SO_4	0.4 M Na_2SO_4	0.2 M Na_2SO_4	0.5 M KCl	0.2 M KCl
E	0.5 M LiCl	0.2 M LiCl	0.5 M RbCl	0.2 M RbCl	0.5 M CsCl	0.2 M CsCl	0.4 M NaF	0.1 M NaF	1.5 M NaBr	0.4 M NaBr	0.1 M NaBr	0.4 M NaI
F	0.1 M NaI	0.4 M $MgCl_2$	5 mM $MgCl_2$	5 mM $CaCl_2$	5 mM $SrCl_2$	1 mM $ZnCl_2$	0.1 mM $ZnCl_2$	1 mM $NiCl_2$	0.1 mM $NiCl_2$	5 mM $MnCl_2$	0.5 mM $MnCl_2$	1 mM $CoCl_2$
G	0.1 mM $CoCl_2$	1 mM $CuSO_4$	0.1 mM $CuSO_4$	1 mM $CdSO_4$	5 mM EDTA pH 7.5	5 mM EGTA pH 7.5	2 mM magic triangle pH 7.0	2 mM $La(NO_3)_3$	2 mM $PrCl_3$	2 mM $NdCl_3$	2 mM $SmCl_3$	2 mM $EuCl_3$
H	2 mM $GdCl_3$	2 mM $DyCl_3$	2 mM $HoCl_3$	2 mM $YbCl_3$	2 mM $LuCl_3$	5 mM Na_2HPO_4	5 mM Na_3VO_4	5 mM Na_2WO_4	5 mM Na_2MoO_4	5 mM DTT	5 mM TCEP pH 7.0	5 mM β-mercapto ethanol

Concentrations shown are final concentrations. The final components of each well will be 1 X screen solution, 10 X SYPRO Orange, and half the original protein concentration.

*NAMI is a free-of-charge program. See Page 5 for further details.



Abbreviations:
EDTA: Ethylenediaminetetraacetic acid; **EGTA:** Ethylene glycol-bis(2-aminoethylether)-*N,N,N',N'*-tetraacetic acid ; **DTT:** 1,4-Dithiothreitol; **TCEP:** Tris(2-carboxyethyl)phosphine hydrochloride; **I3C:** 5-amino-2,4,6-triiodoisopthalic acid

Manufacturer's safety data sheets are available from our website.

Re-Ordering details:

Catalogue Description	Pack size	Catalogue Code
The Durham pH Screen	96 x 0.5 mL	MD1-101
The Durham Salt Screen	96 x 0.5 mL	MD1-102
RUBIC Buffer Screen	96 x 0.5 mL	MD1-96
RUBIC Additive Screen	96 x 0.25 mL	MD1-97
RUBIC Buffer Set*	48 x 11 mL	MD1-96-BUFFER
Single Reagents		
The Durham pH Screen single reagents	96 x 0.5 mL	MDSR-101-well number
The Durham Salt Screen	96 x 0.5 mL	MDSR-102-well number
RUBIC Buffer Screen single reagents	10 mL	MDSR-96-well number
RUBIC Additive Screen single reagents	various volumes	See website for more details.

All stocks are available to buy from Molecular Dimensions.

*The RUBIC Buffer Set contains buffers A1 to B24 at 0.5M buffer, C1 to D24 at 0.5M buffer+ 1.25M NaCl from the RUBIC Buffer Screen.

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