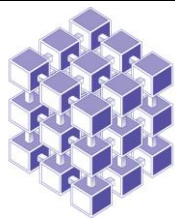
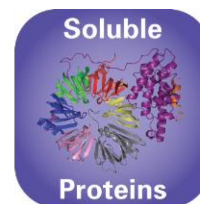




RUBIC Buffer Screen MD1-96

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).

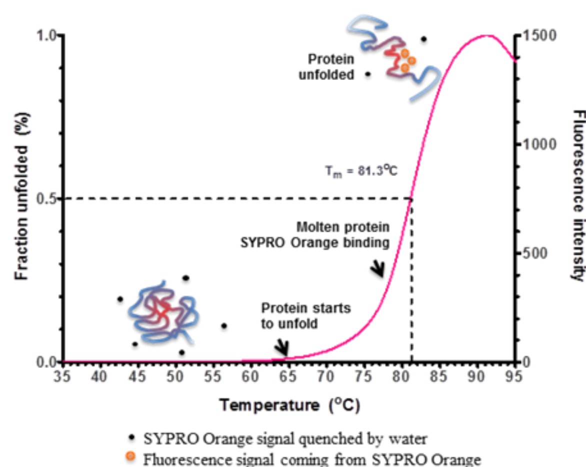
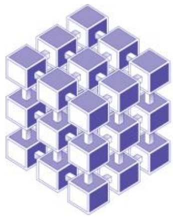
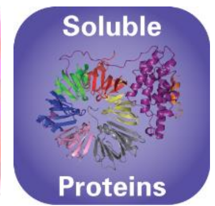


Figure 1

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 µM), glycerol (<10 %), reducing reagent (<5 mM).

- Assay is not compatible with most detergents.

Suggested protocol:

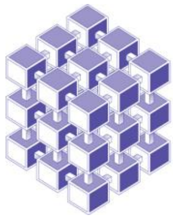
1. Transfer 21 µl of RUBIC Buffer Screen to a PCR-microplate.
2. Spin down the prefilled microplate for 30 seconds.
3. Place the microplate on ice.
4. Dispense in each well 2 µl of the protein. It is strongly advised to use a repeater pipette.
5. Prepare freshly a SYPRO Orange solution (Invitrogen, S6651, 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).

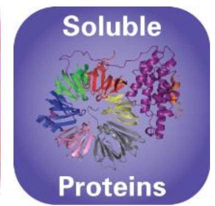
Note: We advise against pre-mixing the protein and the dye. Since the dye contains DMSO, it can damage the protein in higher concentrations or interact with the protein affecting the initial background signal.

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.

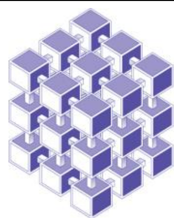
Final pH may vary from that specified on the datasheet. Molecular Dimensions will be happy to discuss the precise formulation of individual reagents.
Individual reagents and stock solutions for optimization are available from Molecular Dimensions.

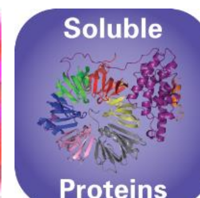
Contact and product details can be found at www.moleculardimensions.com
Enquiries regarding RUBIC Buffer Screen formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

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.

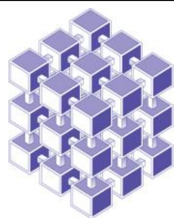
RUBIC Buffer and RUBIC Additive Screens have been designed and developed by Stephane Boivin and Rob Meijers at the EMBL Hamburg and is manufactured exclusively under license by Molecular Dimensions Limited.
Limited Use and Restrictions: Products sold by Molecular Dimensions Ltd. or its affiliates or authorized distributors and information relating to same are intended for research use only in crystal growth and optimization of crystal growth following use of the product by the purchaser and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, including resale or use in manufacture. The license to use RUBIC Buffer Screen and RUBIC Additive Screen specifically excludes any rights to use the product information for the manufacture of the product or derivatives thereof, or distribute, transfer, or otherwise provide access to such information to any third party for any purpose or use.

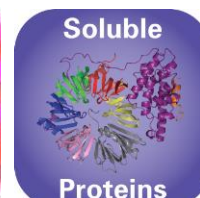




RUBIC Buffer Screen Conditions A1-D12* MD1-96

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 monobasic	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 monobasic	7.0
A11				0.119	M	Potassium phosphate monobasic	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 monobasic	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 monobasic	6.0
C7	0.298	M	Sodium chloride	0.119	M	Citrate	6.0
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 monobasic	7.0
C11	0.298	M	Sodium chloride	0.119	M	Potassium phosphate monobasic	7.0
C12	0.298	M	Sodium chloride	0.119	M	HEPES	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 monobasic	7.5
D5	0.298	M	Sodium chloride	0.119	M	Imidazole	7.5
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

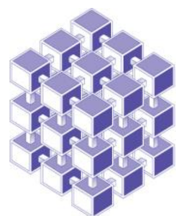




RUBIC Buffer Screen Conditions E1-H12* MD1-96

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 monobasic	7.5
F6				0.06	M	Sodium phosphate monobasic	7.5
F7				0.149	M	Sodium phosphate monobasic	7.5
F8				0.298	M	Sodium phosphate monobasic	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**



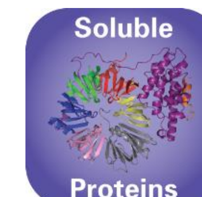
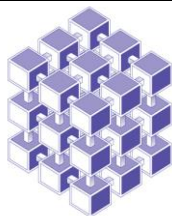
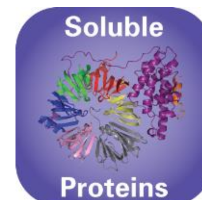


Figure 2:- Layout of the RUBIC Buffer Screen

	1	2	3	4	5	6	7	8	9	10	11	12	
A	Water	100mM Citrate pH 4.0	100mM Na acetate pH 4.5	100mM Citrate pH 5.0	100mM MES pH 6.0	100mM K phosphate monobasic pH 6.0	100mM Citrate pH 6.0	100mM Bis-Tris pH 6.5	100mM MES pH 6.5	100mM Na2 phosphate monobasic pH 7.0	100mM K phosphate monobasic pH 7.0	100mM HEPES pH 7.0	**Buffer and pH screens (low ionic strength)**
B	100mM MOPS pH 7.0	100mM Am acetate pH 7.3	100mM Tris-HCl pH 7.5	100mM Na2 phosphate monobasic 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 Citrate 250mM NaCl pH 4.0	100mM Na acetate 250mM NaCl pH 4.5	100mM Citrate 250mM NaCl pH 5.0	100mM MES 250mM NaCl pH 6.0	100mM K phosphate 250mM NaCl pH 6.0	100mM Citrate 250mM NaCl pH 6.0	100mM Bis-Tris 250mM NaCl pH 6.5	100mM MES 250mM NaCl pH 6.5	100mM Na2 phosphate 250mM NaCl pH 7.0	100mM K phosphate 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 Am acetate 250mM NaCl pH 7.3	100mM Tris-HCl 250mM NaCl pH 7.5	100mM Na2 phosphate 250mM NaCl pH 7.5	100mM Imidazole 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 %w/v 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 Na2 phosphate monobasic pH 7.5	50mM Na2 phosphate monobasic pH 7.5	125mM Na2 phosphate monobasic pH 7.5	250mM Na2 phosphate monobasic 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 Na phosphate / Citrate pH 7.5	50mM MOPS / Na HEPES pH 7.5	0.1M BICINE / Tris 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 monohydrate/Glycine [2:7:7]; **BICINE**: 2-(Bis(2-hydroxyethyl)amino)acetic acid; **MES**: 2-(N-Morpholino)ethanesulfonic acid; **HEPES**: 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid); **MOPS:** 3-(N-Morpholino)propanesulfonic acid; **Tris-HCl**: Trizma® hydrochloride; **Bis-Tris**: 2,2-Bis(hydroxymethyl)-2,2',2"-nitrilotriethanol; **PIPES**: 1,4-Piperazinediethanesulfonic acid; **Bis-Tris propane**: 1,3-Bis[tris(hydroxymethyl)methylamino]propane; **Sodium HEPES**: 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid sodium salt, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) sodium salt; **Tris**: Trizma® base

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
RUBIC Buffer PCR	96 x 21µL	MD1-96-PCR

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

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