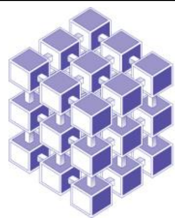
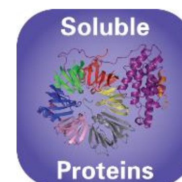




RUBIC Additive Screen MD1-97

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).

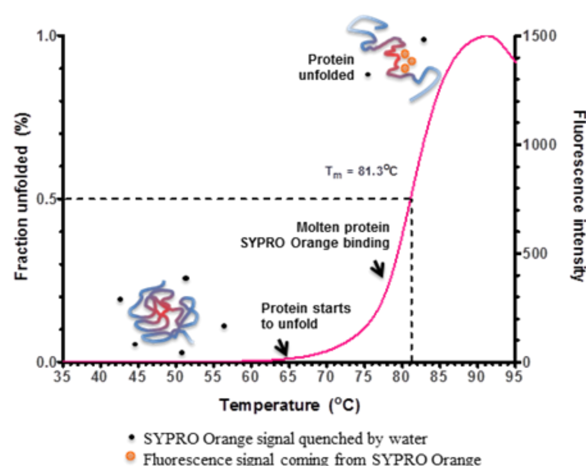
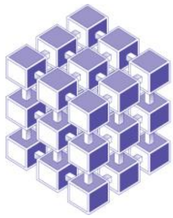
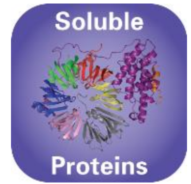


Figure 1

Figure. 1. Typical thermal denaturation assay using Thermofluor.



Molecular Dimensions



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:

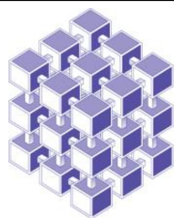
1. Thaw the RUBIC Additive Screen on ice. (Avoid multiple freeze-thaw cycles).
2. Spin down the prefilled microplate for 30 seconds.
3. Transfer 16 µl of RUBIC Additive Screen to a PCR-microplate.
4. Add 5 µl of a 5X buffer. We advise to use a buffer free of salt to prevent competition with reagent from 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)**



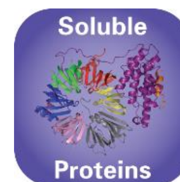


Table 1. Buffers contained in the RUBIC Additive Kit (Tube 1 x 1.5mL water and 23 x 1.5mL buffers).

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.

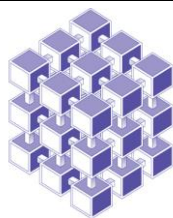
Contact and product details can be found at www.moleculardimensions.com
Enquiries regarding RUBIC Additive Screen formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

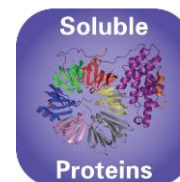
References

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.

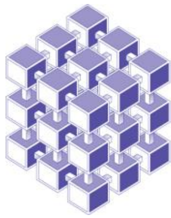
RUBIC Buffer and RUBIC Additive Screens have been designed and developed by Stephane Boivin and Rob Meijers at the EMBL Hamburg, and is manufactured exclusively under license by Molecular Dimensions Limited.
Limited Use and Restrictions: Products sold by Molecular Dimensions Ltd. or its affiliates or 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.

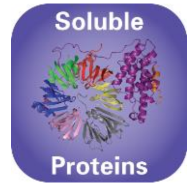




RUBIC Additive Screen Conditions A1-D12 MD1-97

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

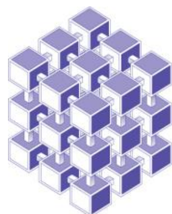




RUBIC Additive Screen Conditions E1-H12 MD1-97

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

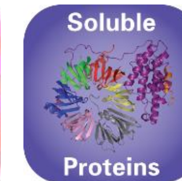
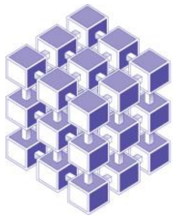


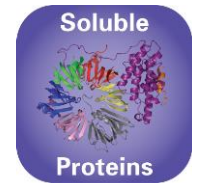
Figure 2. Layout of the of RUBIC Additive Screen

	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