ThermoSOLV-RX™

Protein Thermal Stability Kit

User Guide

Protocol: Identify Formulations that Maximize the Thermal Stability of a Protein Sample

Materials

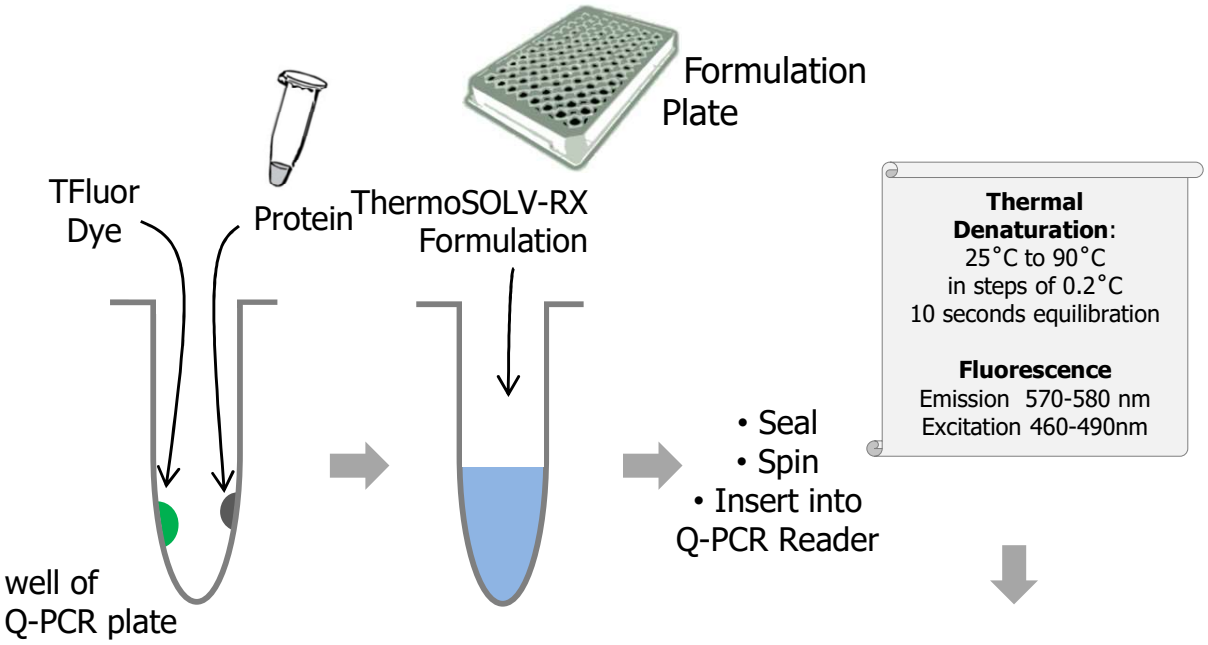
Kit
1 tube TFluor™ Dye
1 Formulation Plate RX (96 x 170 uL)

User-provided
- ca. 100 µL purified protein solution (ca. 1 mg/mL)
- temperature scanning fluorescence plate reader such as a Q-PCR instrument (BioRad CFX, Roche LightCycler® 480, QuantStudio quantitative PCR)
- Q-PCR plate (preferably white)
- plate seal

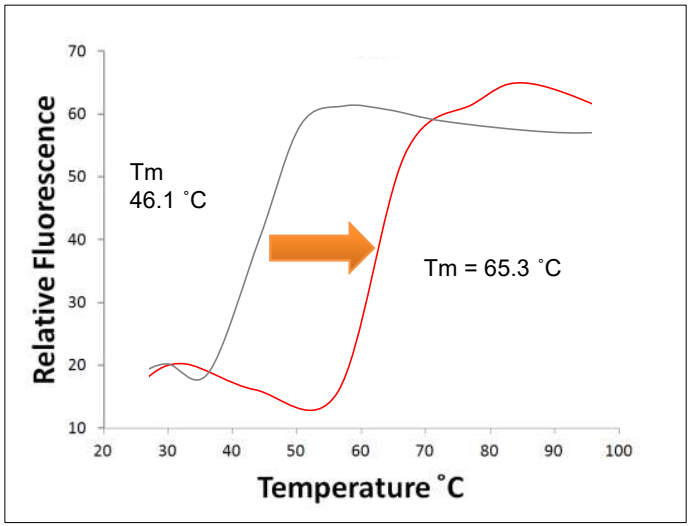
Protocol

1. Dilute 2 µL of TFluor Dye with 200 µL of water and pipette 2 µL of this solution onto the side of each well in a Q-PCR plate.
2. Pipette 1 µL of protein sample into the opposite sides of all wells
3. Add 25 µL of each formulation from the Formulation Plate RX. This combines the protein and the dye in each well.
4. Seal the Q-PCR plate and spin it (*i.e.* 5 min at 1000 rpm) to neatly collect all liquid in the center of the well.
5. Insert the plate into temperature scanning fluorescence plate reader. Run temperature scan (*i.e.* heating from 25˚C to 90˚C in 0.2˚C steps equilibrating for 12 seconds for every step) while recording TFluor fluorescence at 570-580 nm (Excitation at 460-490nm).
6. Analyze the resulting fluorescence / temperature data and record the midpoint of thermal denaturation for each formulation. Compare thermal denaturation points and identify formulation that renders protein most temperature stable.

Variation: include known or putative small molecule ligands or co-factors (ATP, NAD/H, Zn^{2+} etc.) to protein buffer prior to analysis with the OptiTherm kit.



Note & Troubleshooting
We advise to carry out a simple test prior to conducting the ThermoSOLV experiment to dial in the proper protein concentration and to identify a suitable detection range. This can be done by setting up a single well using the amounts suggested in this protocol (using any standard buffer). Consult the manual of the temperature scanning fluorescence plate reader to adjust the fluorescence emission signal to less than 20% of the maximal readout. Increase amounts of protein and dye if fluorescence signal is too low, decrease protein and dye amounts if fluorescence signal is too low.



ThermoSOLV-RX™

Product Information

Content:

- 1 x 96 well Formulation Plate RX
- 1 tube TFluor™ Dye
- Quick Start Guide and MSDS

Store at 4˚C. Caution: TFluor™ Dye not yet fully tested (EU) WGK1 Combustible. Readily absorbed through skin. Target organ(s): Eyes, Skin. Hygroscopic. Product of USA. For R&D use only. Not for drug, household or other uses.

Purpose

ThermoSOLV-RX Protein Thermal Stability Kit

Systematic solution design and fluorescence-based stability assay for:

- **Thermal stabilization of protein samples**

For updated instructions and additional information please refer to www.stablebiologics.com

One ThermoSOLV-RX kit contains consumable materials to assay solution conditions for up to six (6) different protein samples.

Order Information

Order Cat #: SB-004-001

ThermoSOLV-RX Protein Thermal Stability Kit

Price: $ 299 USD (3 pack discounted to $750 USD)

Stable Biologics LLC
1500 1st Ave North
Birmingham, AL 35242
USA

Send order to: cweaver@stablebiologics.com

Reagent Listing

Well #	Row	Col	Buffer#	Conc	unit	pH	Additive NAME	Conc	unit
1	A	1					NaCl	60	mM
2	A	2					Arg/Glu*	100	mM
3	A	3					Arginine-HCl	30	mM
4	A	4					Glycine	100	mM
5	A	5					Poloxamer 188	0.2	%w/v
6	A	6	Acetate	50	mM	5.0	EDTA	4	mM
7	A	7					Na bisulfate	6	mM
8	A	8					Sucrose	100	mM
9	A	9					Sorbitol	100	mM
10	A	10					PEG 400	2	%w/v
11	A	11					Glycerol	6	%w/v
12	A	12	Ammonium sulfate	3	M				
13	B	1					NaCl	60	mM
14	B	2					Arg/Glu*	100	mM
15	B	3					Arginine-HCl	30	mM
16	B	4					Glycine	100	mM
17	B	5					Poloxamer 188	0.2	%w/v
18	B	6	Histidine	50	mM	6.0	EDTA	4	mM
19	B	7					Na bisulfate	6	mM
20	B	8					Sucrose	100	mM
21	B	9					Sorbitol	100	mM
22	B	10					PEG 400	2	%w/v
23	B	11					Glycerol	6	%w/v
24	B	12	DMSO	5	%v/v				
25	C	1					NaCl	60	mM
26	C	2					Arg/Glu*	100	mM
27	C	3					Arginine-HCl	30	mM
28	C	4					Glycine	100	mM
29	C	5					Poloxamer 188	0.2	%w/v
30	C	6	Sodium Succinate	50	mM	6.0	EDTA	4	mM
31	C	7					Na bisulfate	6	mM
32	C	8					Sucrose	100	mM
33	C	9					Sorbitol	100	mM
34	C	10					PEG 400	2	%w/v
35	C	11					Glycerol	6	%w/v
36	C	12	Original sample buffer						
37	D	1					NaCl	60	mM
38	D	2					Arg/Glu*	100	mM
39	D	3					Arginine-HCl	30	mM
40	D	4					Glycine	100	mM
41	D	5					Poloxamer 188	0.2	%w/v
42	D	6	Sodium citrate	50	mM	6.5	EDTA	4	mM
43	D	7					Na bisulfate	6	mM
44	D	8					Sucrose	100	mM
45	D	9					Sorbitol	100	mM
46	D	10					PEG 400	2	%w/v
47	D	11					Glycerol	6	%w/v
48	D	12	Glycine	50	mM	3.0	NaCl	500	mM
49	E	1					NaCl	60	mM
50	E	2					Arg/Glu*	100	mM
51	E	3					Arginine-HCl	30	mM
52	E	4					Glycine	100	mM
53	E	5					Poloxamer 188	0.2	%w/v
54	E	6	Sodium phosphate	50	mM	6.5	EDTA	4	mM
55	E	7					Na bisulfate	6	mM
56	E	8					Sucrose	100	mM
57	E	9					Sorbitol	100	mM
58	E	10					PEG 400	2	%w/v
59	E	11					Glycerol	6	%w/v
60	E	12	Sodium lactate	50	mM	6.5			
61	F	1					NaCl	60	mM
62	F	2					Arg/Glu*	100	mM
63	F	3					Arginine-HCl	30	mM
64	F	4					Glycine	100	mM
65	F	5					Poloxamer 188	0.2	%w/v
66	F	6	Potassium phosphate	50	mM	7.0	EDTA	4	mM
67	F	7					Na bisulfate	6	mM
68	F	8					Sucrose	100	mM
69	F	9					Sorbitol	100	mM
70	F	10					PEG 400	2	%w/v
71	F	11					Glycerol	6	%w/v
72	F	12	Na/K phosphate	50	mM	7.5	Tween 20	0.4	%w/v
73	G	1					NaCl	60	mM
74	G	2					Arg/Glu*	100	mM
75	G	3					Arginine-HCl	30	mM
76	G	4					Glycine	100	mM
77	G	5					Poloxamer 188	0.2	%w/v
78	G	6	Na/K phosphate	50	mM	7.5	EDTA	4	mM
79	G	7					Na bisulfate	6	mM
80	G	8					Sucrose	100	mM
81	G	9					Sorbitol	100	mM
82	G	10					PEG 400	2	%w/v
83	G	11					Glycerol	6	%w/v
84	G	12					Benzyl alcohol	0.2	%w/v
85	H	1					NaCl	60	mM
86	H	2					Arg/Glu*	100	mM
87	H	3					Arginine-HCl	30	mM
88	H	4					Glycine	100	mM
89	H	5					Poloxamer 188	0.2	%w/v
90	H	6	Tris	50	mM	7.5	EDTA	4	mM
91	H	7					Na bisulfate	6	mM
92	H	8					Sucrose	100	mM
93	H	9					Sorbitol	100	mM
94	H	10					PEG 400	2	%w/v
95	H	11					Glycerol	6	%w/v
96	H	12	Tris	50	mM	8.5			

Note:

DMSO: dimethyl sulfoxide. EDTA: ethylenediaminetetraacetic acid. NaCl: sodium chloride. PEG: polyethylene glycol. # pH values for buffers used only; * Arginine-HCl/Glutamic acid, each amino acid is 50 mM
Well A12, B12 and C12 are for control experiments.