OptiTherm™ Quick Start Guide

Protein Thermal Stability Kit

SOLUBLE BIOSCIENCE

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

Materials

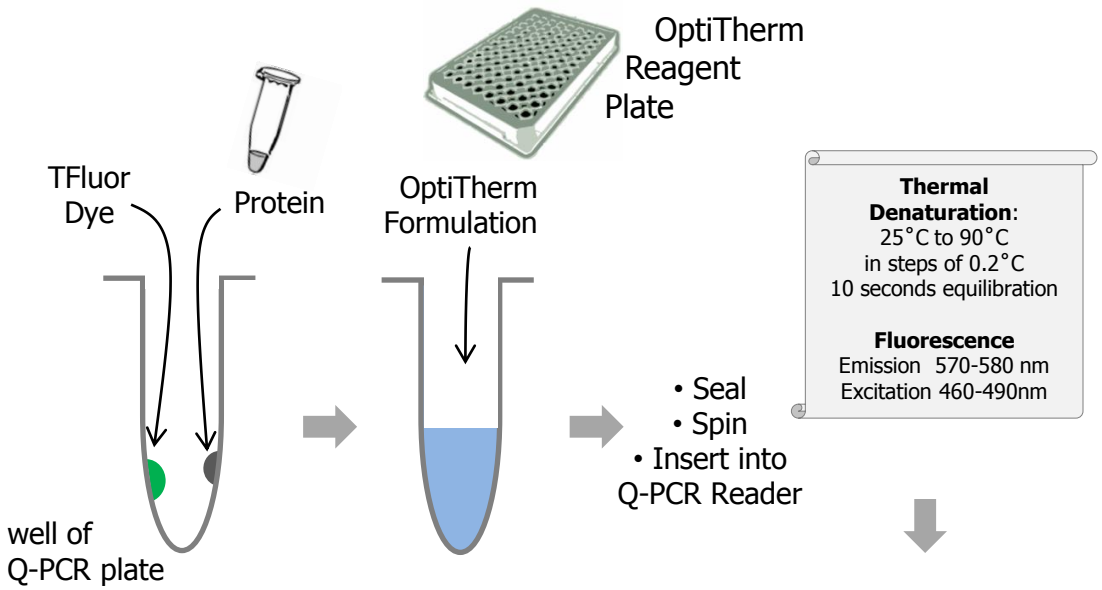
Kit
1 tube TFluor™ Dye
1 OptiReagent Plate (96 x 170 uL)

User-provided
- ca. 100 µL purified protein solution (ca. 1 mg/mL)
- temperature scanning fluorescence plate reader such as a Q-PCR instrument (BioRad Opticon™, Stratagene Mx4000, Roche LightCycler® 480 quantitative PCR)
- Q-PCR plate (preferably white)
- plate seal

Protocol

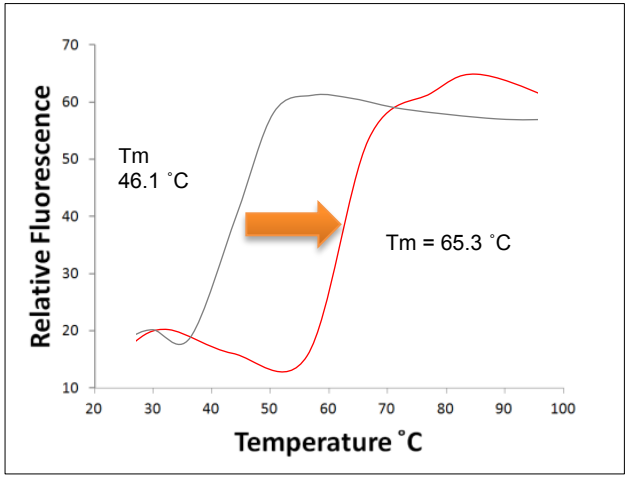
1. Dilute 2 µL of TFluor Dye with 200 µL of water and pipette 2 µL of this solution onto the side of each well in a Q-PCR plate.
2. Pipette 1 µL of protein sample into the opposite sides of all wells
3. Add 25 µL of each formulation from the OptiReagent Plate. This combines the protein and the dye in 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 OptiTherm 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.



OptiTherm

Protein Thermal Stability Kit

Product Information

Content:

- 1 x 96 well OptiReagent Plate
- 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

OptiTherm 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.solublebioscience.com

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

Order Information

Order Cat #: SOL-Therm

OptiTherm Protein Thermal Stability Kit

Price: $ 249 USD (3 pack discounted to $710.00 USD)

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Reagent Listing

Well #	Row	Col	Buffer#	Conc unit	pH	Additive NAME	Additive Conc unit
1	A	1	Glycine	100 mM	3.0		
2	A	2	Citric Acid	100 mM	3.2		
3	A	3	PIPPS	100 mM	3.7		
4	A	4	Citric Acid	100 mM	4.0		
5	A	5	Sodium Acetate	100 mM	4.5		
6	A	6	Na/K Phosphate	100 mM	5.0		
7	A	7	Sodium Citrate	100 mM	5.5		
8	A	8	Na/K Phosphate	100 mM	6.0		
9	A	9	Bis-Tris	100 mM	6.0		
10	A	10	MES	100 mM	6.2		
11	A	11	ADA	100 mM	6.5		
12	A	12	Bis-Tris Propane	100 mM	6.5		
13	B	1	Ammonium Acetate	100 mM	7.0		
14	B	2	MOPS	100 mM	7.0		
15	B	3	Na/K Phosphate	100 mM	7.0		
16	B	4	HEPES	100 mM	7.5		
17	B	5	Tris	100 mM	7.5		
18	B	6	EPPS	100 mM	8.0		
19	B	7	Imidazole	100 mM	8.0		
20	B	8	Bicine	100 mM	8.5		
21	B	9	Tris	100 mM	8.5		
22	B	10	CHES	100 mM	9.0		
23	B	11	CHES	100 mM	9.5		
24	B	12	CAPS	100 mM	10.0		
25	C	1	Glycine	50 mM	3.0	NaCl	150 mM
26	C	2	Sodium Acetate	50 mM	4.5	NaCl	150 mM
27	C	3	Bis-Tris	50 mM	6.0	NaCl	150 mM
28	C	4	MOPS	50 mM	7.0	NaCl	150 mM
29	C	5	Imidazole	50 mM	8.0	NaCl	150 mM
30	C	6	CHES	50 mM	9.5	NaCl	150 mM
31	C	7	Citric Acid	50 mM	3.2	NaCl	500 mM
32	C	8	Na/K Phosphate	50 mM	5.0	NaCl	500 mM
33	C	9	ADA	50 mM	6.5	NaCl	500 mM
34	C	10	HEPES	50 mM	7.5	NaCl	500 mM
35	C	11	Tris	50 mM	8.5	NaCl	500 mM
36	C	12	CAPS	50 mM	10.0	NaCl	500 mM
37	D	1	Glycine	50 mM	3.0	Trehalose	500 mM
38	D	2	Sodium Acetate	50 mM	4.5	Trehalose	500 mM
39	D	3	Bis-Tris	50 mM	6.0	Trehalose	500 mM
40	D	4	MOPS	50 mM	7.0	Trehalose	500 mM
41	D	5	Imidazole	50 mM	8.0	Trehalose	500 mM
42	D	6	CHES	50 mM	9.5	Trehalose	500 mM
43	D	7	Citric Acid	50 mM	3.2	TMAO	500 mM
44	D	8	Na/K Phosphate	50 mM	5.0	TMAO	500 mM
45	D	9	ADA	50 mM	6.5	TMAO	500 mM
46	D	10	HEPES	50 mM	7.5	TMAO	500 mM
47	D	11	Tris	50 mM	8.5	TMAO	500 mM
48	D	12	CAPS	50 mM	10.0	TMAO	500 mM
49	E	1	Glycine	50 mM	3.0	Na_2SO_4	500 mM
50	E	2	Sodium Acetate	50 mM	4.5	Na_2SO_4	500 mM
51	E	3	Bis-Tris	50 mM	6.0	Na_2SO_4	500 mM
52	E	4	MOPS	50 mM	7.0	Na_2SO_4	500 mM
53	E	5	Imidazole	50 mM	8.0	Na_2SO_4	500 mM
54	E	6	CHES	50 mM	9.5	Na_2SO_4	500 mM
55	E	7	Citric Acid	50 mM	3.2	Arg/Glu*	50 mM
56	E	8	Na/K Phosphate	50 mM	5.0	Arg/Glu*	50 mM
57	E	9	ADA	50 mM	6.5	Arg/Glu*	50 mM
58	E	10	HEPES	50 mM	7.5	Arg/Glu*	50 mM
59	E	11	Tris	50 mM	8.5	Arg/Glu*	50 mM
60	E	12	CAPS	50 mM	10.0	Arg/Glu*	50 mM
61	F	1	Glycine	50 mM	3.0	Tween 20	1 % (w/v)
62	F	2	Sodium Acetate	50 mM	4.5	Tween 20	1 % (w/v)
63	F	3	Bis-Tris	50 mM	6.0	Tween 20	1 % (w/v)
64	F	4	MOPS	50 mM	7.0	Tween 20	1 % (w/v)
65	F	5	Imidazole	50 mM	8.0	Tween 20	1 % (w/v)
66	F	6	CHES	50 mM	9.5	Tween 20	1 % (w/v)
67	F	7	Citric Acid	50 mM	3.2	Solubilisin II™	100 % (w/v)
68	F	8	Na/K Phosphate	50 mM	5.0	Solubilisin II™	100 % (w/v)
69	F	9	ADA	50 mM	6.5	Solubilisin II™	100 % (w/v)
70	F	10	HEPES	50 mM	7.5	Solubilisin II™	100 % (w/v)
71	F	11	Tris	50 mM	8.5	Solubilisin II™	100 % (w/v)
72	F	12	CAPS	50 mM	10.0	Solubilisin II™	100 % (w/v)
73	G	1	Glycine	50 mM	3.0	Glycerol	20 % (w/v)
74	G	2	Sodium Acetate	50 mM	4.5	Glycerol	20 % (w/v)
75	G	3	Bis-Tris	50 mM	6.0	Glycerol	20 % (w/v)
76	G	4	MOPS	50 mM	7.0	Glycerol	20 % (w/v)
77	G	5	Imidazole	50 mM	8.0	Glycerol	20 % (w/v)
78	G	6	CHES	50 mM	9.5	Glycerol	20 % (w/v)
79	G	7	Citric Acid	50 mM	3.2	Betaine	1 M
80	G	8	Na/K Phosphate	50 mM	5.0	Betaine	1 M
81	G	9	ADA	50 mM	6.5	Betaine	1 M
82	G	10	HEPES	50 mM	7.5	Betaine	1 M
83	G	11	Tris	50 mM	8.5	Betaine	1 M
84	G	12	CAPS	50 mM	10.0	Betaine	1 M
85	H	1	H2O	100 %			
86	H	2	H2O	100 %			
87	H	3					
88	H	4				AmSulfate	3 M
89	H	5				Acetonitrile	80 % (v/v)
90	H	6	PEG 1450	10 %		NaCl	50 mM
91	H	7				DTT	1 mM
92	H	8				DTT	5 mM
93	H	9				DTT	15 mM
94	H	10				BME	2.5 mM
95	H	11				BME	10 mM
96	H	12				BME	20 mM

TMAO, Trimethylamine N-Oxide; PIPPS, Piperazine-N, n'-Bis (3-Propanesulfonic Acid); MES, 2-(N-morpholino) ethanesulfonic acid; MOPS, 3-(N-morpholino) propanesulfonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Arg/Glu*: 50mM of each Arginine and Glutamate; DDT, DL-Dithiothreitol; BME, 2-Mercaptoethanol; Betaine, Trimethyl-Glycine; CAPS, N-cyclohexyl-3-aminopropanesulfonic acid; ADA, N-(2-Acetamido)iminodiacetic Acid; Tris, tris(hydroxymethyl)aminomethane; CHES, 2-(N-Cyclohexylamino)ethane Sulfonic Acid; EPPS, N-(2-hyroxyethyl)piperazine-N'-(3-propanesulfonic acid). # pH values for buffers used only; * each amino acid is 50 mM