OptiTherm™ Protein Thermal Stability Kit Quick Start Guide

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

Materials

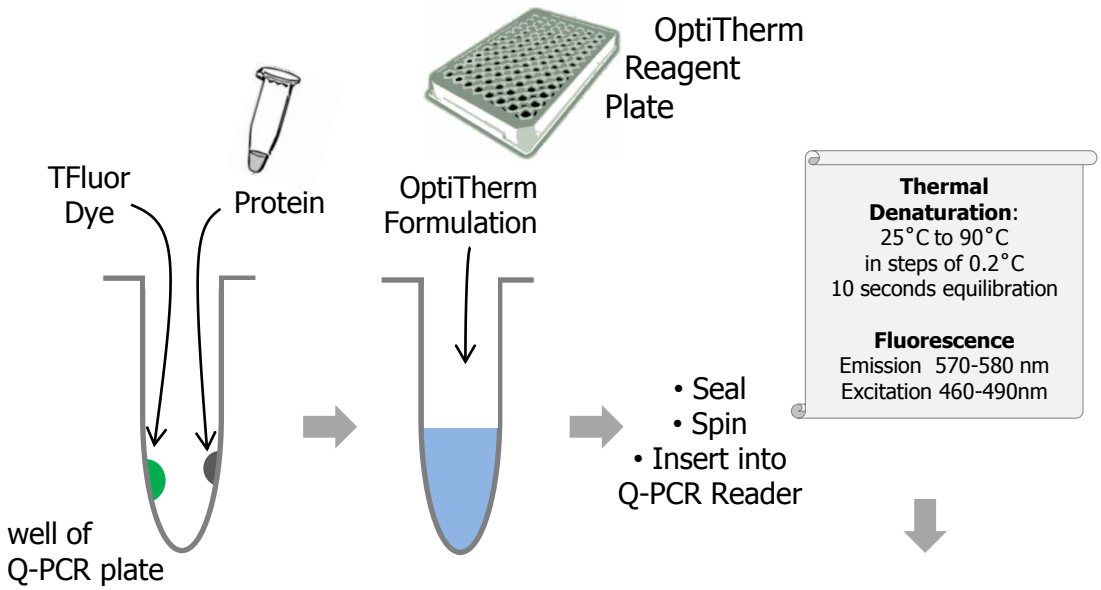
Kit
1 tube TFluor™ Dye
1 OptiTherm Formulation Plate (96 x 150 uL)

User-provided
- ca. 100 uL 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

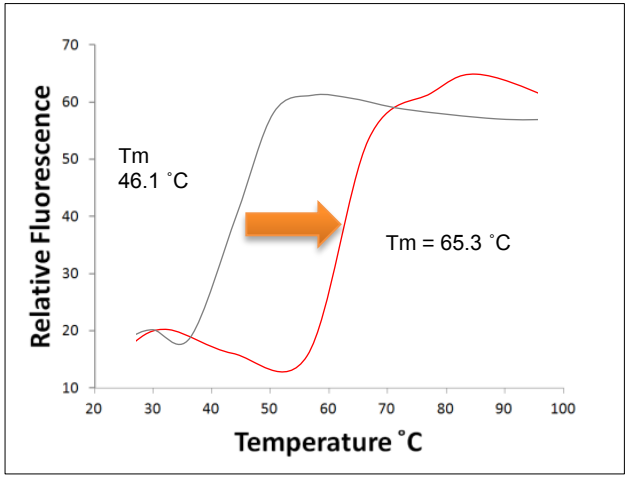
Protocol

1. Dilute 1uL of TFluor Dye with 100 uL water and pipette a 2 uL drop of this solution onto the side of each well in a Q-PCR plate
2. Pipette 1 uL protein sample into the opposite sides of all wells
3. Add 25 uL of each formulation from the OptiTherm Formulation Plate. This combines the protein with the dye in each well.
4. Seal the Q-PCR plate and spin (*i.e.* 5 min at 1000 rpm) to neatly collect all liquid in the center of the well
5. Insert 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 460-490nm)
6. Analyze 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 OptiTherm Reagent Plate
- 1 tube TFluor™ Dye
- Quick Start Guide with OptiTherm Reagent Listing

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. MSDS available www.dilyx.com/optitherm. 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 check: www.dilyx.com/optitherm

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

Order Information

Order #: DLX-104-006

OptiTherm Protein Thermal Stability Kit

Price: $ 599 (inquire about volume discounts)

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DILYX

OptiTherm Reagent Listing

Well #	Row	Col	Buffer#	Conc	unit	pH	Additive NAME	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	1.0	M
38	D	2	Sodium Acetate	50	mM	4.5	Trehalose	1.0	M
39	D	3	Bis-Tris	50	mM	6.0	Trehalose	1.0	M
40	D	4	MOPS	50	mM	7.0	Trehalose	1.0	M
41	D	5	Imidazole	50	mM	8.0	Trehalose	1.0	M
42	D	6	CHES	50	mM	9.5	Trehalose	1.0	M
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

Well #	Row	Col	Buffer#	Conc	unit	pH	Additive NAME	Conc	unit
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™	100	% (w/v)
68	F	8	Na/K Phosphate	50	mM	5.0	Solubilisin™	100	% (w/v)
69	F	9	ADA	50	mM	6.5	Solubilisin™	100	% (w/v)
70	F	10	HEPES	50	mM	7.5	Solubilisin™	100	% (w/v)
71	F	11	Tris	50	mM	8.5	Solubilisin™	100	% (w/v)
72	F	12	CAPS	50	mM	10.0	Solubilisin™	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	2	M
80	G	8	Na/K Phosphate	50	mM	5.0	Betaine	2	M
81	G	9	ADA	50	mM	6.5	Betaine	2	M
82	G	10	HEPES	50	mM	7.5	Betaine	2	M
83	G	11	Tris	50	mM	8.5	Betaine	2	M
84	G	12	CAPS	50	mM	10.0	Betaine	2	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					DDT	1	mM
92	H	8					DDT	5	mM
93	H	9					DDT	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