

OptiTherm™ Quick Start Guide

Protein Thermal Stability Kit



Predictive Oncology®

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

Materials

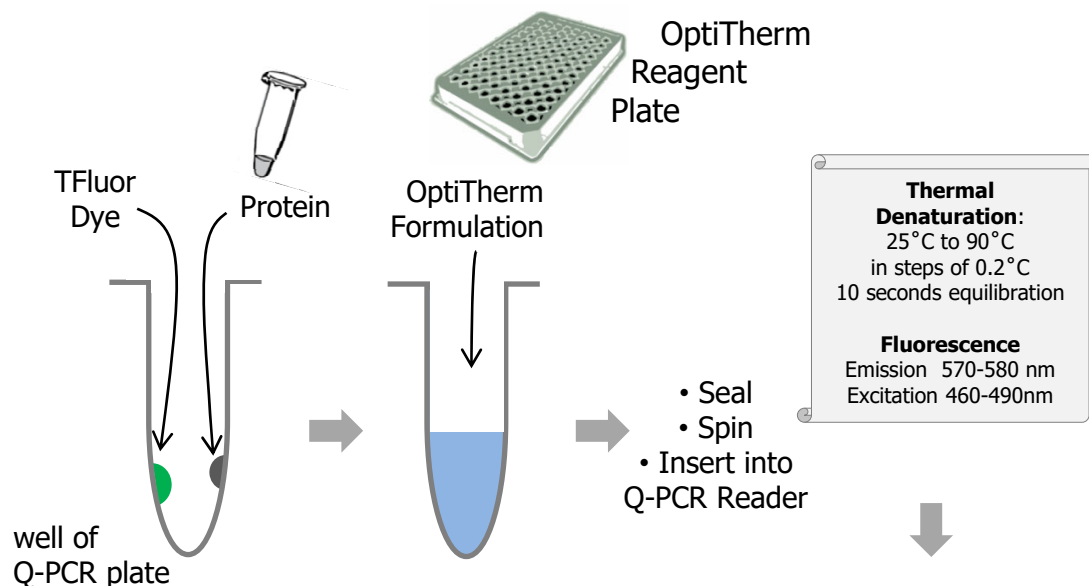
- Kit**
- 1 tube TFluor™ Dye
 - 1 OptiReagent Plate (96 x 170 uL)
- User-provided**
- ca. 100 µL purified protein solution (ca. 1 mg/mL)
 - temperature scanning fluorescence plate reader such as a Q-PCR instrument (BioRad Opticon™, Stratagene Mx4000, Roche LightCycler® 480 quantitative PCR)
 - Q-PCR 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.

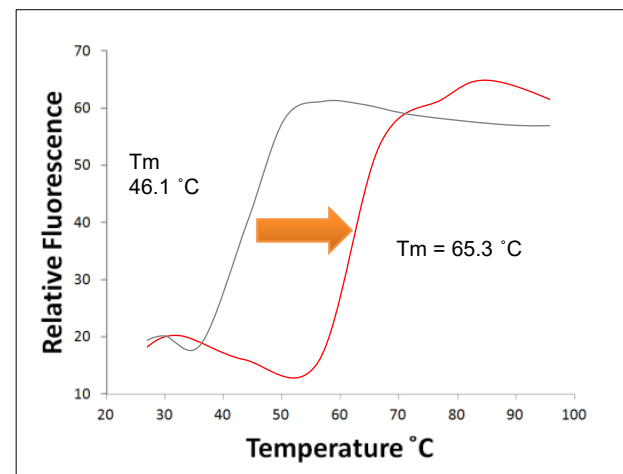
Variation: include known or putative small molecule ligands or co-factors (ATP, NAD/H, Zn²⁺ etc.) to protein buffer prior to analysis with the OptiTherm kit.

BioRad Opticon™ is a trademark of Bio-Rad Laboratories, Inc.
Stratagene Mx4000™ is a trademark of Life Technologies Corporation
Roche LightCycler® is a trademark of is a trademark of Roche Diagnostics Corporation



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.predictive-oncology.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: \$ 399 USD (3 pack discounted to \$999 USD)

Predictive Oncology
200 Riverhills Business Park
Ste. 250
Birmingham, AL 35242
www.predictive-oncology.com
orders@predictive-oncology.com
USA

Reagent Listing

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