OptiSol™

Protein Solubility Screening Kit Application Manual

Dilyx **OptiSol** Protein Solubility Screening Kit. Systematic solution design and array-based filtration technology that enables to either

→ identify formulations that **protect** a target protein **from aggregation**

or to

→ gently **solubilize** an **aggregated protein** sample

The accompanying **Protein Dashboard™** aids in spotting protein behavior trends and identify the critical solvent factors for optimal protein solubilization within a single experiment.

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Application Manual
Vb1.1

OptiSol™

Protein Solubility Screening Kit

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A copy of this Application Manual is available online at www.dilyx.com/resources
Protein Dashboard™ can be downloaded online at www.dilyx.com/resources.
We thank you for supporting us in minimizing printed materials shipped with our products, and making our Earth a better place.

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OptiSol™ Protein Solubility Screening Kit

Application Manual: Table of Contents

Introduction

Use this Application Manual to find out how to use the OptiSol Protein Solubilization Screening Kit to find the *'solubility sweet spot'* for a particular protein or to *'solubilize reversibly aggregated protein'*.

Proteins aggregate. This undesired process is often caused by elevated temperature, vigorous stirring, addition of ligands or molecular binding partners or just by mere storage of a protein sample over a period of time. Protein aggregation can often be avoided with proper choice of pH, salt or stabilizing additives. This OptiSol Protein Solubilization Screening Kit contains a systematically varied array of buffers (from pH 3 to pH 10) and a series of solubility enhancers (salts, amino acids, sugars, polyols, reducing reagents) that enable the determination of conditions under which a particular protein sample is protected from aggregation or can be de-aggregated. A total of 90 different formulations with solubility enhancers can be tested in one single, label-free experiment; the remaining 6 experiments are positive and negative control experiments.

Soluble proteins pass filters, aggregated proteins don't.
This simple feature is exploited to in the filtration step. The wells that contain soluble proteins will yield protein after the filtration process, those with protein aggregates will not contain any protein.

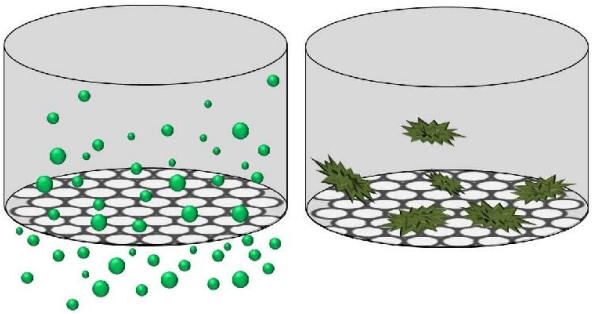


Figure 1 *Filtration principle: soluble proteins pass the filter due to their smaller size (left), while aggregated proteins do not pass filter (right).*

The OptiSol Protein Solubility Screening Kit Protocols can be applied to soluble or aggregated protein samples and subjected to the processes outlined below:

PROTOCOL A: Protein Solubility Profiling

Starting from non-aggregated protein sample

1. Dilute solubilized protein sample in each of the supplied formulation
2. Challenge with aggregation inducing situation (heat, time, ligand …)
3. Filter the solution
4. Assess the filtrate (solution that passes the MWCO filter); the filtrate containing most solubilized protein has the optimal solubilization formulation

PROTOCOL B: Aggregate Solubilization

Starting from reversibly aggregated protein sample

1. Dilute aggregated protein sample in each of the supplied formulation
2. Filter the solution
3. Assess the filtrate (solution that passes the MWCO filter); the filtrate containing most solubilized protein has the optimal solubilization formulation (Tab. 6)

Detailed Protocols are described on p14 (Protocol A) & p18 (Protocol B).

Analysis of Results. We advise to select a sensitive protein detection assay to characterize the filtrate, preferentially in 96-well format. In most cases plate reader data can be directly copy-and-pasted into the **Protein Dashboard™** Excel Spreadsheet to evaluate the results of the experiment and obtain graphical support tosspot protein behavior trends and identify the critical solvent factors for optimal protein solubilization.

Principle of the Kit

Depending on the desired information and the nature of the starting sample, the OptiSol™ Protein Solubilization screening kit can be used for two different purposes: solubilization of protein aggregate (Protocol B) or identification of conditions that maintain the protein soluble when a certain stress is applied (Protocol A).

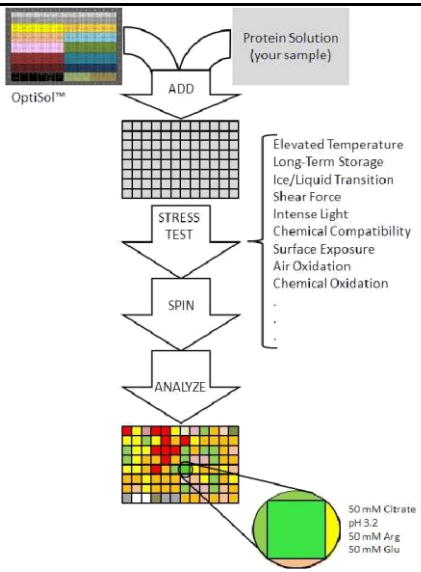
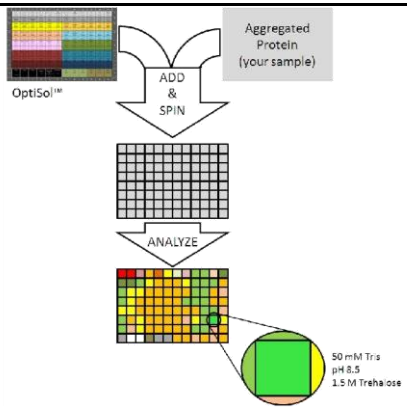
Protocol A: Protein Solubility Profiling	Protocol B: Solubilize Aggregated Protein
	
Result: optimal formulation (pH, salt, etc.) that maintains protein sample solubility under given stress	***Result:*** optimal formulation (pH, salt, etc.) that converts aggregated protein into soluble form

Figure 2 *Two different OptiSol Protocols that can be applied to a protein sample with the OptiSol Protein Solubilization Screening kit. Protocol A utilizes a protein solution and identifies optimized conditions that maintain the protein in solution, despite application of a certain stress. Protocol B utilizes reversibly aggregated protein and identifies conditions under which an aggregated protein sample becomes solubilized.*

Table 1 *Examples for experimental stresses that can be applied to protein solutions after they are mixed with the OptiSol formulations.*

Type of Stressor	Example for Experimental Stress Test and Parameters
Elevated Temperature	Incubate 24 hours at 37°C
Long-Term Storage	Store 2 weeks at room temperature
Ice/Liquid Transition	Freeze and thaw 20 times
Shear Force	Force material 20 x through narrow syringe need
Intense Light	Expose samples to direct sunlight or UV light for 1 h
Chemical Compatibility	Add 10 mM of caustic reagent (i.e. heavy metal)
Surface Exposure	Add 5 uL of 10 um diameter glass beads
Air Oxidation	Bubble 10 ml of air through sample
Chemical Oxidation	Add Hydrogen Peroxide

Table 2 *Examples for diagnostic analytical tests that may be applied to determine the quantity or activity of protein in the filtered solution. The various assays may be based on detection of Fluorescence or Radioactivity (Protein Assay, enzyme assay, western/ELISA, ligand binding assay). Note that assays may require compensation for OptiSol formulations or may not work in the presence of the OptiSol formulations. Please consult the appropriate assay manual or utilize OptiSol formulations as controls.*

Analysis Technique	Experimental Result
UV/Vis Absorption	Protein quantity
Fluorescence	Protein quantity, functional activity etc.
Protein Assay (Bradford, BCA…)	Protein quantity
Enzyme Assay	Functional activity, specific enzymatic activity
Western, Dot Blot/ELISA	Immunological binding quantity
Binding Assay	Functional activity, specific ligand binding activity
DLS (Dynamic Light Scattering)	Sample homogeneity/polydispersity, hydrodynamic radius

Kit Components

Reagents Provided and Kit Storage

Storage at 4°C is recommended. Consumption of the OptiSol™ Protein Solubility Kit is recommended within 4 weeks of receipt. Avoid repeated freezing and thawing. All reagents are provided as solutions, each 200 uL in wells within a 96-well based format, sufficient to subject one protein sample to one 96-well based solubility assay.

OptiSol Kit Components

The OptiSol™ Solubility Screening Kit contains the following items:

Table 3 *Components and quantities of the OptiSol™ Solubility Screening Kit*

QTY	***ITEM***
1	OptiSol™ Reagent Plate
1	Reaction Plate
1	Filter Plate
1	Collection Plate
1	Tech Sheet

OptiSol™ Reagent Plate

Reagent plate filled with 96 x 160 uL reagents. Each well is filled with 160 uL solution and capped. The composition of each well is described below in Table 4.
Note that the pH is systematically varied from pH 3 to pH 10 with buffer types overlapping in the neutral and slightly acidic or slightly basic region. In addition to pH variation, ionic strength, organic solvents, amino acids, detergents, sugars, polyols and reducing reagents are present. The systematic variation of pH and solubility enhancers (Additives) allow for a systematic assessment of the solubility behavior.
Positive controls (#85-87) as well as negative controls (#87-90) are included in the OptiSol™ Reagent Plate (see Table 5)

Table 4: Reagents list for OptiSol™ Reagent Plate

Well		Buffer#			Additive	
#	Row Col		Conc unit	pH	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

pH values for buffers used only; Arg/Glu*: each amino acid is 50mM
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.

Well			Buffer[#]			Additive	
#	Row	Col		Conc unit	pH	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

pH values for buffers used only; Arg/Glu*: each amino acid is 50mM
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).

Table 5 *Controls included in the OptiSol™ Reagent Plate*

Negative Controls (no solubilization enhancement)	**Positive Controls (strong precipitation)**
Water only – no sample (H1)	3 M Ammonium sulfate (H4)
Water only (H2)	80% Acetonitrile (H5)
Empty (H3)	10% PEG1500, 50 mM NaCl (H6)

Filter Plates

The OptiSol™ Protein Solubility Screening kit is compatible with a wide diversity of protein particle sizes ranging from <1 kDa to 30 kDa and from >30kDa to 200 kDa. Filter plates should be selected according to the expected MW of the solubilized protein particle (Table 6; also see Filter Plate selection guide, Appendix page 22).

Table 6 *Filter Plates available for the OptiSol™ Protein Solubility Screening kit*

Filter Plate MWCO 30	**Filter Plate MWCO 200**
Peptides or proteins <30kDa when monomeric	Proteins < 200 kDa when monomeric
Oligomeric protein complexes with total molecular weight < 30 kDa	Oligomeric protein complexes with total molecular weight < 200 kDa

Collection Plate

Collection Plate with 96 wells x max. 250 uL

Additional Materials Required

Pipettors with disposable plastic tips capable of pipetting 1-20 uL and 20-200uL preferred. We recommend the use of 8 or 12-channel adjustable precision P20 and P200 pipettors.

Protein sample. Subjecting a protein or peptide sample to the OptiSol kit requires sufficient protein sample quantities. The protein quantity and concentration needs to be matched to the assay that is used to detect the protein. This quantity (and concentration) depends on the particular application and the protein detection assay used (see below). For example, if each OptiSol formulation is combined with 20 uL of protein sample, a total of 96 x 20 uL = ca. 2 mL is required to use the kit.

It is advisable to test the assay with protein quantities that are desired to be detected with a single sample.

Protein detection assay. A suitable assay to detect fractions of the applied target protein within each well is required. Applicable assays include SDS-PAGE, Western blot, ELISA, specific UV or Fluorescence-based detection or other ways to establish the presence of the particular target protein. Since the protein sample is divided into 96 portions, the protein assay needs to detect protein at such small quantities. For instance, if one subjects 96 x 20 uL and the target protein concentration is 100 ug/mL, the assay is required to detect fractions of the target protein – in this example in the range of 2 ug to 0.1 ug. In other words, the assay should allow to detect the target protein at a higher than 1:10 dilution that of the initial sample concentration within a volume of 10 uL or less.

Centrifuge with swing-bucket rotor that can hold and spin the 96-well block plate stack assembly (Filter Plate + Collection Plate).

Safety Warnings and Precautions

- The OptiSol™ Protein Solubility Kit is sold for RESARCH USE ONLY.

- Do not use this reagent kit in humans, do not use it for diagnosis of humans, do not use it as a drug.

- Handle the materials contained in the kit with due care and exercise attention when using the kit.

- Components of this kit may contain hazardous substances. Reagents can be harmful if ingested or absorbed through the skin and may cause irritation to the eyes. Reagents should be treated as possible mutagens and should be handled with care and disposed of properly.

- Observe good laboratory hygiene and practices. Always use gloves, wear a lab coat and protective eyewear. Never pipet by mouth. Do not eat, drink or smoke in the laboratory.

- To minimize oxidation, store kit at 4°C and use kit within 4 weeks of receipt.

Methods and Procedures

The OptiSol Protein Solubility Screening kit can be used for two fundamentally different purposes (also see "Principle of the Kit"):

1. **Protein Solubility Profiling (Protocol A)**
2. **Solubilize an Aggregated Protein Sample (Protocol B)**

Both Applications are described in the following Protocols:

PROTOCOL A:

PROTEIN SOLUBILITY PROFILING

Use this protocol for samples of soluble protein to analyze solubility and protect from aggregation. Choose a particular aggregation stress such as elevated temperature, or freeze-thawing to establish and analyze aggregation behavior (see Table 1 for more sample stresses). The OptiSol™ Protein Solubility Screening kit will yield information on solution conditions that yield minimized aggregation when exposed to a particular stress.

Preparation

TEMPERATURE. *Allow all reagents to assume equal temperature (4°C or room temperature). For best temperature control equilibrate all kit solutions in a temperature-controlled 96-well block. The use of a PCR Thermal Cycler set at a constant temperature is recommended.*

REAGENTS. *Make sure that all reagents are free of any crystallization. If crystals are observed, incubate at room or elevated temperature for several hours until inorganic crystals are dissolved*

SAMPLE AMOUNT. *Make sure that sufficient protein sample is available to distribute into 95 equal portions. Typical sample requirements are 1-20 uL of sample / well (requiring 100 uL – 2 mL of protein solution). To assay different conditions, each sample is diluted by a factor of 2 – 10. This dilution needs to be taken into consideration when choosing an appropriate assay (see below).*

PROTEIN ASSAY. *Make sure to have an assay to measure the amount of solubilized target protein. This Protocol is designed to utilize ca. 1 mg of aggregated protein sample, where the total protein concentration is 1 mg/mL and an assay will be applied that can detect quantities down to ca. 0.1 ug of protein.*

FILTER PLATE. *Select proper Filter Plate by matching expected particle size with MWCO of Filter Plate. Consult Filter Plate selection guide Table 6 or 9, Appendix page 22.*

PROCESS CONSIDERATION. *If large quantities of protein solution are available, subject at first only a fraction of the protein to this OptiSol solubilization protocol. This allows to first identify optimized solubilizing conditions and then, in a second step, to transfer the protein into a larger volume of the stabilizing solution.*

Protein Solubility Profiling

NOTE: *This protocol requires 2 mL of protein solution: during the course of the protocol, one hundred aliquots (20 uL) are diluted each 10-fold. The protocol can be scaled down however, simply by reducing the volume of added protein (i.e. 1 uL, requiring less than 100 uL of protein sample). The protein concentration should be as high as possible.*

If necessary, protein volumes in each well may be increased to 100 uL (note though, that the concentration of the solubilizing buffer will be drastically different from that shown in Table 4.

*In this **Protein Solubility Profiling Protocol** the stress test applied is elevated temperature and 1 day storage. Select a different stress test as desired (consult Table 1).*

1. Remove caps from OptiSol plate and transfer 150 uL from each well into the corresponding well of the Reaction Plate. Add to each well 20 uL of protein solution by slowly pipetting the volume while swirling the pipette tip in the solution. Seal plate with tape.

2. Apply the stress to test aggregation behavior. For instance, store overnight at 37°C.

3. Transfer 160 uL from each well (except well H3, which is filled with only 20 uL) into the corresponding well of the Filter Plate (see Filter Plate selection guide in Table 6 or 9, Appendix page 22).

4. Assemble Filter Plate Stack by combining the Collection Plate (bottom) with the Filter Plate on top. Check orientation (well A1 of filter plate should match well A1 of the collection Plate). Insert Filter Plate Stack into swing-bucket of rotor, add counter weight as balance and spin at room temperature for ca. 15 min at 3,000 rpm.

5. Disassemble Filter Plate Stack and visually inspect if all wells (note: well H3 may contain only very little liquid) in Collection Plate are filled with liquid.

6. Subject each well of Collection Plate to target protein specific assay (see Table 1 for options). *I.e.* use 50 uL of each well for a Bradford assay

Evaluation

Transfer quantitative data of protein specific assay for each well to the ProteinDashboard™ (www.dilyx.com/optisol/proteindashboard) spreadsheet to obtain a visual summary of the result. This can often be done simply by a cut-and-paste operation from a plate-reader output file into the ProteinDashboard™. The wells with the highest protein levels indicate OptiSol™ solutions that maintain the protein solubilized in the presence of the stress. Such protein samples can be used for further experimentation. Note positive controls (H1, H2, H3) and negative controls (H4, H5, H6) as specified in section Table 5.

If the OptiSol™ Protein Solubilization Screening assay was carried out with only a fraction of available material, the remaining protein solution may be protected against a particular stress by transferring the protein solution into the identified optimal solubilization solution.

Expected Results

The aggregation behavior of most proteins is highly dependent on the pH. Typically their solubility is low and their tendency to aggregate is maximal in the region of the protein's isoelectric point. Often the nature of the particular buffer contributes to the solubility of a protein at a particular pH (Chan & Warwicker, 2009; Jancarik et al., 2004).

Proteins that aggregate by forming disulfide bridges don't aggregate in the presence of DTT (H7-9) or beta-mercapto ethanol (H10-12).

Elevated salt concentrations have been shown to 'salt in' proteins, hence increasing their solubility (Jenkins, 1998).

Additives such as detergent, glycerol, TMAO, Arginine and Glutamate, Trahalose and Betaine have been shown to enhance protein solubility. Their mechanism of action is not always well understood (Arakawa et al., 2006 & 2007).

PROTOCOL B:

SOLUBILIZE AN AGGREGATED PROTEIN SAMPLE

Use this protocol for reversibly aggregated protein samples. Note that some protein aggregation is irreversible and can therefore usually not be properly solubilized. The OptiSol™ Protein Solubilization kit can be used however, to assess if further attempts to de-aggregate a particular protein sample are of use (*i.e.* consider giving up on an aggregated protein sample if the OptiSol™ kit does not yield any solubilized protein).

Preparation

TEMPERATURE. *Allow all reagents to assume equal temperature (4°C or room temperature). For best temperature control equilibrate all kit solutions in a temperature-controlled 96-well block. The use of a PCR Thermal Cycler set at a constant temperature is recommended.*

REAGENTS. *Make sure that all reagents are free of any crystallization. If crystals are observed, incubate at room or elevated temperature for several hours until inorganic crystals are dissolved.*

SAMPLE AMOUNT. *Make sure that sufficient protein sample is available to distribute into 95 equal portions.*

PROTEIN ASSAY. *Make sure to have an assay capable of measuring the amount of solubilized target protein. This Protocol is designed to utilize 1 mg of aggregated protein sample, where the total protein concentration is 1 mg/mL and an assay will be applied that can detect less than 0.1 ug of protein.*

FILTER PLATE. *Select proper Filter Plate by matching expected particle size with MWCO of Filter Plate. Consult with Filter Plate selection guide Table 6 or 9, Appendix page 22.*

PROCESS CONSIDERATION. *If large quantities of aggregated protein solution are available, subject at first only a fraction of the aggregated protein to this OptiSol solubilization protocol. This allows to first identify solubilizing conditions and then, in a second step, scale up the solubilization of the entire aggregated protein solution.*

Solubilization of an Aggregated Protein Sample

1. Prepare homogenized sample from aggregated protein solution. If a pellet is present, disrupt pellet by (bath) ultra sonication, douncing or repeated aspiration and dispensation until sample is homogenously clear or opaque.
2. Remove caps from OptiSol plate and pipette to each well 10 uL of homogenized protein aggregate solution.
3. Attach caps, mix by vortexing and incubate at room temperature for ca. 10 min.
4. Collect liquid by short spin in the centrifuge or by gently hitting the plate onto the lab bench. Transfer 160 uL from each well (except well H3, which is filled with only 10 ul) into the filter plate (see Filter Plate selection guide in Table 6 or 9, Appendix page 22).
5. Assemble Filter Plate Stack by combining the Collection Plate (bottom) with the Filter Plate on top. Check orientation (well A1 of filter plate should match well A1 of the collection Plate). Insert Filter Plate Stack into swing-bucket of rotor, add counter weight as balance and spin at room temperature for ca. 15 min at 3,000 rpm.
6. Disassemble Filter Plate Stack and visually inspect if all wells in Collection Plate are filled with liquid (note: well H3 may contain only very little liquid).
7. Subject each well of Collection plate to target protein specific assay (see Table 1 for options). *I.e.* use 50 uL of each well for a Bradford assay.

Evaluation

Transfer quantitative data of protein assay for each well to ProteinDashboard™ spreadsheet (www.dilyx.com/optisol/proteindashboard) to inspect a visual summary of the result. This can often be done simply by a cut-and-paste operation from a plate-reader output file into the ProteinDashboard™. The wells with the highest level protein levels indicate OptiSol™ solutions that can solubilize the aggregated protein sample. Such protein samples can be used for further experimentation. Note that some protein aggregate is irreversibly denatured and can therefore not be properly solubilized.

If the OptiSol™ protein aggregate solubilization was carried out with only a fraction of available material, the remaining aggregated protein solution may solubilized by scaling up the solubilization reaction in a linear fashion.

Expected Results

The solubility of most proteins is highly dependent on the pH. Typically, solubility is high in pH regions distant from the protein's isoelectric point. Furthermore, the nature of the particular buffer often contributes to the solubility of a protein at a particular pH (Chan & Warwicker, 2009; Jancarik et al., 2004).

Proteins that aggregate by forming disulfide bridges may be solubilized by reduction with DTT (H7-9) or BME (H10-12).

Elevated salt concentrations have been shown to 'salt in' proteins, hence increasing their solubility (Jenkins, 1998).

Additives such as detergent, glycerol, TMAO, Arginine and Glutamate, Trehalose and Betaine have been shown to enhance protein solubility. Their mechanism of action is not always well understood (Arakawa et al., 2006 & 2007).

Supplemental Protocols

Proteins aggregate when exposed to certain stresses, and the modulation of this aggregation behavior in the presence of a variety of reagents and pH values can be analyzed with the OptiSol™ kit. Select from one of the Stresses listed in Table 7 and incorporate the stress into the assay to analyze the protein's aggregation behavior.

Table 7 *Examples for experimental stresses that can be applied to protein solutions after they are mixed with the OptiSol formulations.*

Type of Stressor	Example for Experimental Stress Test and Parameters
Elevated Temperature	Incubate 24 hours at 37°C
Long-Term Storage	Store 2 weeks at room temperature
Ice/Liquid Transition	Freeze and thaw 20 times
Shear Force	Force material 20 x through narrow syringe need
Intense Light	Expose samples to direct sunlight or UV light for 1 h
Chemical Compatibility	Add 10 mM of caustic reagent (i.e. heavy metal)
Surface Exposure	Add 5 uL of 10 um diameter glass beads
Air Oxidation	Bubble 10 ml of air through sample
Chemical Oxidation	Add Hydrogen Peroxide

APPENDIX

Filter Plate Selection

The OptiSol™ Protein Solubility Screening kit is available for two different protein particle sizes as described below.

Table 8 *Product Order Information*

Product	Protein Size	Catalog Number
OptiSol™ Protein Solubility Kit 30	< 30 kDa	DLX-102-030
OptiSol™ Protein Solubility Kit 200	< 200 kDa	DLX-102-200

Select the Filter Plate according to the expected molecular weight of the protein particle.

MWCO 30 Filter Plate:

If proteins are monomeric, simply classify according to molecular weight (MW) according to Table 9. For instance, a 23kDa monomeric protein should be assayed with a MWCO 30 plate; and a 90 kDa monomeric protein should be assayed with a MWCO 200 plate.

MWCO 200 Filter Plate:

If protein forms a complex (*i.e.* a homo trimer consisting of three 23 kDa proteins) the MWCO 200 plate should be chosen.

Table 9 *Filter Plates available for the OptiSol™ Protein Solubility Screening kit*

Filter Plate MWCO 30	Filter Plate MWCO 200
Peptides or proteins <30kDa when monomeric	Proteins < 200 kDa when monomeric
Oligomeric protein complexes with total molecular weight < 30 kDa	Oligomeric protein complexes with total molecular weight < 200 kDa

References

Arakawa,T., Philo,J.S., Ejima,D., Tsumoto,K., Sato,H., Arisaka, F. (2006 & 2007) Aggregation analysis of therapeutic proteins; parts 1, 2, 3. *Bioprocess International* 4 (10) : 32-42; 5 (4) : 36-47; 5 (10) : 52-70.

Chan, P., Warwicker, J. (2009)
Evidence for the adaptation of protein pH-dependence to subcellular pH
BMC Biology, 7:69.

Jancarik *et al.* (2004)
Optimum solubility (OS) screening: an efficient method to optimize buffer conditions for homogeneity and crystallization of proteins.
Acta Cryst. D 60:1670.

Jenkins, W.T. (1998)
Three solutions of the protein solubility problem.
Protein Sci. 7(2):376-82.

Troubleshooting Guide

PROTOCOL A: Protein Solubility Profiling

1. No protein detected in any wells

Either there was not enough protein introduced into the assay (A) and hence, no protein was detected, or all of the protein aggregated (B).

A) Check with positive controls H2 and H3 if sufficient quantities of protein were applied. Consider further concentrating the protein sample prior to subjecting to OptiSol™.

B) Decrease the severity of stress applied to the protein sample for by shortening the time of exposure to stress, apply fewer repetitions of freeze-thaw cycles etc.

C) Wrong selection of Filter plate. Check with Table 9 if proper Filter Plate type was used. Consider repeating with the larger MWCO Filter Plate.

2. All wells have similar protein amounts

A) The stress applied may not have been severe enough. Increase the severity of stress applied to the protein sample by increasing the duration of exposure to the stress, apply more repetitions of freeze-thaw cycles, increase the temperature etc.

B) The protein concentration may have been too low to form aggregates. Increase the concentration of the protein sample subjected to the OptiSol™ assay.

C) Wrong selection of Filter plate. Check with Table 9 if proper Filter Plate type was used. Consider repeating with the smaller MWCO Filter Plate

PROTOCOL B: SOLUBILIZE AN AGGREGATED PROTEIN SAMPLE

1. No protein detected in wells

Either there was not enough protein introduced into the assay and hence, no protein was detected, or the protein sample applied was irreversibly aggregated and could not be solubilized.

A) Consider increasing the protein quantity prior to subjecting to the OptiSol™ assay.

B) Wrong selection of Filter plate. Check with Table 9 if proper Filter Plate was used. Consider repeating with larger MWCO Filter Plate.

2. All wells have similar protein amounts

A) The protein may de-aggregate by simple dilution into any of the liquids provided in the assay.

B) Decrease the protein quanitity applied to the OptiSol™ assay.

C) Wrong selection of Filter plate. Check with Table 9 if proper Filter Plate type was used. Consider repeating with the smaller MWCO Filter Plate

Product Warranty

Dilyx Biotechnologies, LLC does not offer any warranty, expressed or implied on the OptiSol™ Protein Solubility Kit. The suitability of this kit for a particular protein target and sample has to be assessed by the user. The chemical compatibility of formulations provided with the OptiSol™ Protein Solubility Kit with that of the target sample is the responsibility of the customer. No Guarantee is made that your target protein will be solubilized or that optimized solubility conditions can be determined when applying this kit.

Product Limitations

The OptiSol™ Protein Solubility Kit is sold for RESARCH USE ONLY. Do not use this reagent kit in humans, do not use it for diagnosis of humans, do not use it as a drug. Handle the materials contained in the kit with due care and exercise attention when using the kit. Any commercial use, development or exploitation of the kit or technical development using this kit without written authorization by Dilyx Biotechnologies, LLC is strictly prohibited.

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Shipping

Telephone orders received Monday through Friday before 12 (noon) Pacific Time are typically shipped on the same day if reagent kit is available in inventory. Continental U.S.A. orders are shipped via FedEx two-day delivery or FedEx Priority Overnight. Please indicate with your order other preferred shipping methods. International orders are shipped via FedEx Priority, delivery typically within 2 – 5 business days. Freight costs to be prepaid and added to invoice.

F.O.B. Seattle, WA, U.S.A.

Technical Support and Returns

Please inspect package upon receipt and contact Dilyx Biotechnologies immediately of any damage or issues; we will replace damaged products at no cost to you.

Please contact us at support@dilyx.com if you have any questions regarding our products. We are happy to discuss any inquiries that you may have

Order Information

OptiSol™ Protein Solubility Screening Kit

All orders received by Dilyx Biotechnologies, LLC will be fulfilled according to the Dilyx Standard Term and Conditions, available at: www.dilyx.com/terms_and_conditions.

Where to place an order:

Fax: (202) 207-0398
Email: support@dilyx.com
Website: www.dilyx.com

Please provide the following when placing an order:

- Name of institution and – if set up – your account number
- Purchase Order number (PO#)
- Catalog number(s) or names of products with quantity of item(s):

Product	Protein Size	Catalog Number
OptiSol™ Protein Solubility Kit 30 **OptiSol™ Protein Solubility Kit 200**	**< 30 kDa** **< 200 kDa**	**DLX-102-030** **DLX-102-200**

- Billing address
- Shipping address (if separate from billing address)
- Contact

Application Manual Vb1.1

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