OptiPharma™

Protein Solubility Screening Kit
Application Manual

OptiPharma 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

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

The accompanying **Pharma 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

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A copy of this Application Manual is available online at www.solublebioscience.com
Pharma Dashboard™ can be downloaded online at www.solublebioscience.com.
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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APPLICATION MANUAL: TABLE OF CONTENTS

Introduction

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

Protein aggregates. This undesired process is often caused by elevated temperature, vigorous stirring, addition of ligands or molecular binding partners, or 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. OptiPharma Protein Solubility Screening Kit contains a combination of 10 types of buffers (pH varies from 3 to 8.5) and 13 pharmaceutical excipients (salts, amino acids, sugars, surfactants, preservatives). A total of 93 different formulations in a 96-well format can be tested in single, label-free experimental setup. The remaining 3 wells are for control experiments.

Soluble proteins pass filters, aggregated proteins don't.
This simple feature is exploited 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.

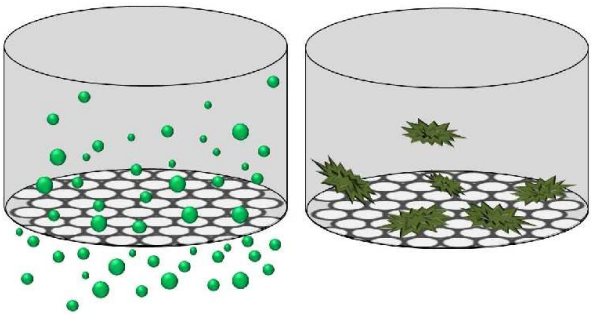

Figure 1 *Filtration principle: soluble proteins pass the filter due to their smaller size (left), while aggregated proteins do not pass the filter (right).*

The OptiPharma 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, etc.)
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

Detailed Protocols are described on page 15 (Protocol A) and page 19 (Protocol B).

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

Principle of the Kit

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

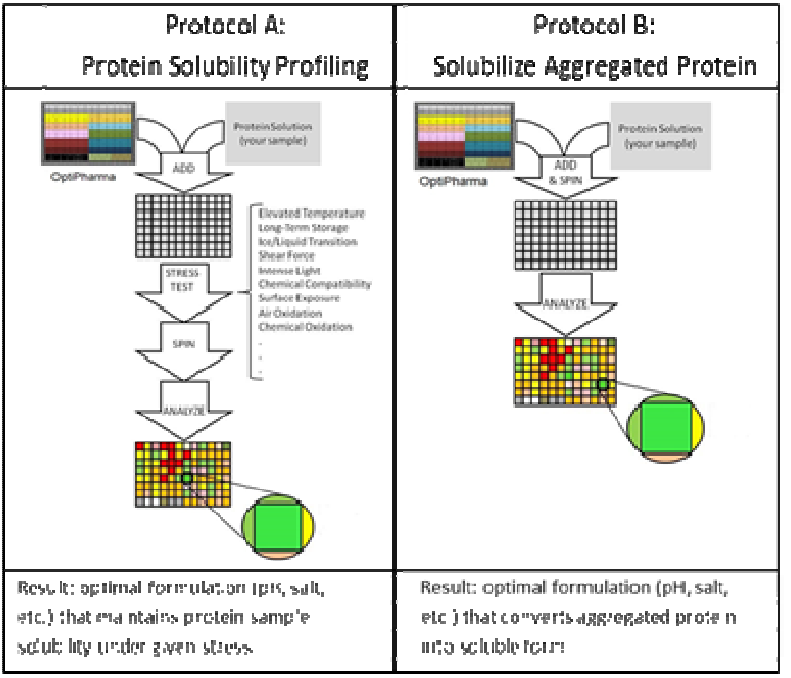

Figure 2 *Two different OptiPharma Protocols that can be applied to a protein sample using the OptiPharma Protein Solubilization Screening kit. Protocol A utilizes a protein solution and identifies optimized conditions that maintain the protein in solution, despite application of a certain stress. Protocol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

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

Type of Stressor	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 times through narrow syringe needle
Intense Light	Expose samples to direct sunlight or UV light for 1 h
Chemical Compatibility	Add 10 mM of caustic reagent
Surface Exposure	Add 5 µL of 10 µm diameter glass beads
Air Oxidation	Bubble 10 mL of air through sample
Chemical Oxidation	Add hydrogen peroxide

Table 2 *Examples of 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 OptiPharma formulations or may not work in the presence of the OptiPharma formulations. Please consult the appropriate assay manual or utilize OptiPharma 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 OptiPharma™ Protein Solubility Kit is recommended within 4 weeks of delivery. Avoid repeated freezing and thawing. All reagents are provided as solutions, each 175 µL in wells of a 96-well based format, sufficient to subject one protein sample to one 96-well based solubility assay.

OptiPharma Kit Components

The OptiPharma™ Solubility Screening Kit contains the following items:

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

QTY	***ITEM***
1	OptiPharma™ Formulation Plate
1	Reaction Plate
1	Filter Plate
1	Collection Plate
1	Quick start guide

OptiPharma™ Formulation Plate

The composition of each well is described in Table 4. OptiPharma Protein Solubility Screening Kit contains a combination of 10 types of buffers (pH varies from 3 to 8.5) and 13 pharmaceutical excipients (salts, amino acids, sugars, surfactants, preservatives). Three wells serve as controls. They are well A12 (ammonium sulfate), B12 (DMSO), and C12 (empty well for sample original buffer).

Table 4: Reagents list for OptiPharma™ Formulation Plate

Well			Buffer[#]				Additive		
#	Row	Col		Conc	unit	pH	NAME	Conc	unit
1	A	1	Acetate	50	mM	5.0	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					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					Dextrose	100	mM
11	A	11					Glycerol	6	%w/v
12	A	12	Ammonium sulfate	3	M				
13	B	1	Histidine	50	mM	6.0	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					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					Dextrose	100	mM
23	B	11					Glycerol	6	%w/v
24	B	12	DMSO	5	%v/v				
25	C	1	Sodium Succinate	50	mM	6.0	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					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					Dextrose	100	mM
35	C	11					Glycerol	6	%w/v
36	C	12	Original sample buffer						
37	D	1	Sodium citrate	50	mM	6.5	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					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					Dextrose	100	mM
47	D	11					Glycerol	6	%w/v
48	D	12	Glycine	50	mM	3.0	NaCl	500	mM

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

Well			Buffer#				Additive		
#	Row	Col		Conc	unit	pH	NAME	Conc	unit
49	E	1	Sodium phosphate	50	mM	6.5	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 18	0.2	%w/v
54	E	6					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					Dextrose	100	mM
59	E	11					Glycerol	6	%w/v
60	E	12	Sodium lactate	50	mM	6.5			
61	F	1	Potassium phosphate	50	mM	7.0	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 18	0.2	%w/v
66	F	6					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					Dextrose	100	mM
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	Na/K phosphate	50	mM	7.5	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 18	0.2	%w/v
78	G	6					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					Dextrose	100	mM
83	G	11					Glycerol	6	%w/v
84	G	12					Benzyl alcohol	0.2	%w/v
85	H	1	Tris	50	mM	7.5	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 18	0.2	%w/v
90	H	6					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					Dextrose	100	mM
95	H	11					Glycerol	6	%w/v
96	H	12	Tris	50	mM	8.5			

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

Filter Plates

The OptiPharma™ Protein Solubility Screening kit is compatible with a wide diversity of protein sizes ranging from several kDa to 250 kDa. Filter plates should be selected according to the expected MW of the solubilized protein molecule (Table 5; also see Filter Plate selection guide, Appendix page 23).

Table 5 *Filter Plates available for the OptiPharma™ Protein Solubility Screening kit*

Optisol Type	Size
OptiPharma I	Peptides, size < 10 kDa
OptiPharma II	Small protein molecules > 10 kDa and < 25 kDa
OptiPharma III	Protein molecules up to 90 kDa
OptiPharma IV	Large protein molecules including antibodies and oligomers up to 250 kDa

Collection Plate and Reaction Plate

Collection or Reaction Plate with 96 wells, each well has a maximum volume of 250 µL.

Additional Required Materials

Pipettors with disposable plastic tips capable of pipetting 1-20 µL and 20-200 µL are 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 OptiPharma kit requires sufficient protein sample quantities. The

protein quantity or concentration has to be matched to the assay that is used to detect the protein. This quantity (or concentration) depends on the particular application and the protein detection assay used (see below). For example, if each OptiPharma formulation is combined with 15 µL of protein sample, a total of 96 x 15 µL = ca. 2 mL is required to use with the kit. Note that the protein will be diluted when mixed with OptiPharma formulations. In this example, 15 µL/(15 + 150) µL or about 11 times dilution. Thus, the original protein concentration has to be 11 times the final protein concentration in the OptiPharma formulation.

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 µL and the target protein concentration is 100 µg/mL, the assay is required to detect fractions of the target protein – in this example in the range of 2 µg to 0.1 µg. 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 µL 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 OptiPharma™ 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 3 months of receipt.

Methods and Procedures

The OptiPharma 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 OptiPharma™ 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.*

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

SAMPLE AMOUNT. *Make sure that sufficient protein sample is available to distribute into 96 equal portions. Typical sample requirements are 1-20 µL of sample / well (requiring 100 µL – 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 µg of protein.*

FILTER PLATE. *Select proper Filter Plate by matching expected particle size with MWCO of Filter Plate. Consult Filter Plate selection guide Table 5 (page 11) or 8 (Appendix page 22).*

PROCESS CONSIDERATION. *If large quantities of protein solution are available, subject at first only a fraction of the protein to this OptiPharma 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 µL) are diluted each 10-fold. The protocol can be scaled down however, simply by reducing the volume of added protein (i.e. 1 µL, requiring less than 100 µL of protein sample). The protein concentration should be as high as possible.*

If necessary, protein volumes in each well may be increased to 100 µL (note 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 if desired (consult Table 1).*

1. Add 180 µL of storage formulation (the buffer used to dissolve the protein) into well H3 of the OptiPharma Formulation plate. Remove caps from formulation plate and transfer 150 µL from each well into the corresponding well of the Reaction Plate. Add to each well 20 µL 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 µL from each well into the corresponding well of the Filter Plate (see Filter Plate selection guide in Table 5 (page 11) or 8 (Appendix page 22).

4. Assemble a Filter Plate Stack by combining the Collection Plate at the bottom and 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 a counter weight as balance and spin at room temperature for 30 min at 3,000 rpm.

5. Disassemble Filter Plate Stack and visually inspect if all wells in Collection Plate are filled with liquid.

6. Subject each well of Collection Plate to target protein specific assay (see Table 1 for options). For example, use 50 µL of each well for a Bradford assay. An established method using UV280 for detection is described in Appendix page 23.

Evaluation

Transfer quantitative data of protein specific assay for each well to the Pharma Dashboard™ 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 Pharma Dashboard™. The wells with the highest protein levels indicate OptiPharma™ solutions that maintain the protein solubilized in the presence of the stress. Such protein samples can be used for further experimentation.

If the OptiPharma™ 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 at maximum 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).

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

Additives such as detergent, sugars, salts, amino acids have been shown to enhance protein solubility.

PROTOCOL B:

SOLUBILIZE AN AGGREGATED PROTEIN SAMPLE

Use this protocol for reversibly aggregated protein samples. Note that some protein aggregation is irreversible, therefore may not be properly solubilized. The OptiPharma™ Protein Solubilization kit can be used however, to assess if further attempts to de-aggregate a particular protein sample is possible (*i.e.* consider giving up on an aggregated protein sample if the OptiPharma™ 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.*

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

SAMPLE AMOUNT. *Make sure that sufficient protein sample is available to distribute into 96 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 µg 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 5 (page 11) or 8 (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 OptiPharma solubilization protocol. This allows to first identify solubilizing conditions, then 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 ultra-sonication, or repeated aspiration and dispensation until sample is homogenously clear or opaque.
2. Remove caps from OptiPharma Formulation plate. Add 180 µL of storage formulation (the buffer in which the aggregated protein is suspended) into well H3 of the formulation plate. Pipette to each well 10 µL 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. Transfer 160 µL from each well into the filter plate (see Filter Plate selection guide in Table 5 (page 11) or 8 (Appendix page 22).
5. Assemble a Filter Plate Stack by combining the Collection Plate at the bottom and 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 30 min at 3,000 rpm.
6. Disassemble Filter Plate Stack and visually inspect if all wells in Collection Plate are filled with liquid.
7. Subject each well of Collection plate to target protein specific assay (see Table 1 for options). For example, use 50 µL of each well for a Bradford assay.

Evaluation

Transfer quantitative data of protein assay for each well to Pharma Dashboard™ spreadsheet 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 Pharma Dashboard™. The wells with the highest level protein levels indicate OptiPharma™ 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, therefore may not be properly solubilized.

If the OptiPharma™ protein aggregate solubilization was carried out with only a fraction of available material, the remaining aggregated protein solution may be 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).

Elevated salt concentrations have been shown to 'salt in' proteins, hence increasing their solubility (Jenkins, 1998).
Additives such as detergent, sugars, salts, amino acids have been shown to enhance protein solubility.

Supplemental Protocols

Proteins aggregate when exposed to certain stresses. The modulation of this aggregation behavior in the presence of a variety of reagents and pH values can be analyzed with the OptiPharma™ kit. Select from one of the Stresses listed in Table 6 and incorporate the stress into the assay to analyze the protein aggregation behavior.

Table 6 *Examples for experimental stresses that can be applied to protein solutions after they are mixed with the OptiPharma 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 times through narrow syringe needle
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 µL 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 OptiPharma™ Protein Solubility Screening kit is available for four different ranges of protein sizes as described below. Each of these kits is also available in triplicate set.

Table 7 *Product Order Information*

Product	Catalog Number
OptiPharma™ Protein Solubility Kit I	SOL-Pharm-I
OptiPharma™ Protein Solubility Kit II	SOL-Pharm-II
OptiPharma™ Protein Solubility Kit III	SOL-Pharm-III
OptiPharma™ Protein Solubility Kit IV	SOL-Pharm-IV

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

Table 8 *Filter Plates available for the OptiPharma™ Protein Solubility Screening kit*

Optisol Type	**Size**
OptiPharma I	Peptides, size < 9 kDa
OptiPharma II	Small protein molecules > 10 kDa and < 25 kDa
OptiPharma III	Protein molecules up to 90 kDa
OptiPharma IV	Large protein molecules including antibodies and oligomers up to 250 kDa

Protocol on Protein Solubility Profiling with UV280 as Detection Method

1. Fill well H3 of OptiPharma formulation plate with 175 µL of storage formulation (formulation in which the protein is dissolved before use in OptiPharma experiment).

2. Transfer 100 µL from each well into the corresponding well of a half-width UV280 plate. We use Greiner Bio-one plates #675801. Scan the plate. This result is used as blank measurement. Transfer the 100 µL of each formulation back into the original Reagent Plate.

3. Transfer 150 µL from each well of the Reagent Plate into the corresponding well of the Reaction Plate. Add 15 µL of protein solution to each well by slowly pipetting the volume while swirling the pipette tip in the solution. Seal plate with tape. Refer to the note on page 24 on preparing the suitable protein concentration.

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

5. Transfer all liquid from each well of the Reaction Plate to the corresponding well of the Filter Plate (see Filter Plate selection guide in Table 5 (page 11) or 8 (Appendix page 22).

6. Assemble Filter Plate Stack by combining the Collection Plate at the bottom and 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 30 min at 3,000 rpm.

7. Disassemble Filter Plate Stack and visually inspect if all wells

in Collection Plate are filled with liquid.

8. Transfer 100 µL from each well of Collection plate into the corresponding well of a half-width UV280 plate, note the wells with less than 100 µL of volume for analysis purpose.

9. Measure the UV280 absorbance, subtract the values with the corresponding blank values collected in step 2.

10. Analyze the data to determine the best formulations for solubilizing the protein of interest. Use the Pharma Dashboard for a quick overview of the result.

Note: Preparing the suitable protein concentration

The protein concentration will be diluted by 11 times (15 µL/165 µL) in the reaction plate when the protein solution is combined with the reagent. Thus, one has to start with a stock protein solution that is 11 times the final solution concentration in the reaction plate. About 2 mL of protein stock solution is adequate. The UV280 absorbance in half-width UV280 wells has to be at least 0.4 (absorbance units). In this case we use half-width UV280 plate from Greiner Bio-one plates (#675801).

The required protein concentration depends on the extinction coefficient of the protein and the sensitivity of the detection assay.

References

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Aggregation analysis of therapeutic proteins; parts 1, 2, 3.
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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) hence no protein was detected, or all of the protein aggregated (B).

A) Check with control wells A12, B12, C12 if sufficient quantities of protein were applied. Consider further concentrating the protein sample prior to subjecting to OptiPharma Formulations.

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 8 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 OptiPharma™ assay.

C) Wrong selection of Filter plate. Check with Table 8 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 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 OptiPharma™ assay.

B) Wrong selection of Filter plate. Check with Table 8 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 quantity applied to the OptiPharma™ assay.

C) Wrong selection of Filter plate. Check with Table 8 if proper Filter Plate type was used. Consider repeating with the smaller MWCO Filter Plate.

Product Warranty

SolubleBioScience Inc. does not offer any warranty, expressed or implied on the OptiPharma™ 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 OptiPharma™ 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 OptiPharma™ 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 SolubleBioScience Inc. 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 USA orders are shipped via UPS or Fedex. Please indicate with your order if you have preferred shipping methods. International orders are shipped via FedEx/UPS Priority, delivery typically within 2 – 5 business days. Custom fees and taxes are the responsible of the customer.

Technical Support and Returns

Please inspect package upon receipt and contact SolubleBioScience Inc. immediately of any damage or issues. We will replace damaged products at no cost to you.

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

Order Information

OptiPharma™ Protein Solubility Screening Kit

All orders received by SolubleBioScience Inc. will be fulfilled according to the company Standard Term and Conditions, available at: www.solublebioscience.com

Where to place an order:

Email: info@solublebioscience.com
Website: www.solublebioscience.com

Please provide the following when placing an order:

- Name of institution and – if set up – your account number
- Purchase Order number (PO#)
- Catalog numbers or names of products with quantity of item(s).
- Billing address
- Shipping address (if separate from billing address)
- Contact information.

Application Manual

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