Optimum Solubility Screen Assay (OSS) Standard Operating Procedure (SOP)

Based on: Lepre, C. A., Moore, J. M.; Microdrop screening: A rapid method to optimize solvent conditions for NMR spectroscopy of proteins; Journal of Biomolecular NMR, 12: 493-499, 1998.

Reference: Jancarik, J., Pufan, R., Hong, C., Kim, R., Kim, S.-H. Optimum Solubility (OS) Screening: an efficient method to optimize buffer conditions for homogeneity and crystallization of proteins. Acta Cryst. D60): 1670-1673.

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Materials/Reagents/Equipment	Vendor
Disposables	Hampton Research, Aliso Viejo, CA 1-800-452-3899
VDX 24 well plate	Hampton Research #HR3-140
Glass cover slips	Hampton Research #HR3-211
Cryschem Plate (sitting drop)	Hampton Research #HR3-158
Reagents	
24 buffers (Used at 0.1 M concentration) Additives (as needed)	All buffers are made using chemicals from Fluka, Calbiochem, and Research Organics. The buffers are filter sterilized and stored at 4^{o} C except for Buffer #3 (PIPPS) which must be stored at room temperature.
Equipment	
Dyna Pro 99 Dynamic Light Scattering (DLS) instrument	Wyatt Technology Corporation, Santa Barbara, CA 1-805-681-9009

Purpose

Try to find a buffer that will disaggregate the protein of interest. This assay is for proteins that cannot be concentrated and/or have poor DLS data.

Procedure

* **Buffer Screening**

___ 1. Get the protein as concentrated as possible. **Protein sample should be at least 10 mg/ml.**

___ 2. Grease a 24 VDX 24 well plate.

___3. Aliquot 500 µl of each buffer (0.1 M concentration) to the wells. Buffer #1 in well 1, buffer #2 in well 2, etc.

___4. Place a glass cover slip on a dark surface for better visibility. Pipette 1 µl of buffer #1 from the reservoir on the glass cover slip.

___5. **With a new pipette tip**, add 1 µl of your protein sample to the buffer drop.

___6. Flip over the glass cover slip and firmly apply onto the corresponding greased buffer well plate. Be careful not to break the glass cover as you place it onto the plate.

___7. Repeat steps 4-6 for all 24 wells.

___7 a. If the protein cannot be concentrated to 10 mg/ml, use a sitting drop plate and place 7 µl of protein + 7 µl of buffer onto the post of a Cryschem Plate (Hampton HR3-158), add 500 µl of reservoir into the well. After overnight incubation at room temperature, collect all 14 µl and read in DLS instrument.

___8. Let the plate incubate overnight at room temperature.

___9. Observe the clarity of the drops under a microscope and score each drop on the OSS data sheet as follows**:**

C = clear drops **P** = precipitated drops

___10. Select conditions that are absolutely clear and perform DLS on those conditions. Take the drop (<2 µl) and add the corresponding buffer [14 µl] to generate a DLS sample of 15 µl. You want the final protein concentration of the DLS sample to be 1 – 2 mg/ml (the minimum concentration to obtain accurate results is 1 mg/ml) and the minimum volume of the sample is 15 µl.

___11. Save the 24 well plate for the next OSS. Do not discard the old glass slips until you need the plate again to eliminate buffer evaporation. When reusing, just re-grease the plate and use new glass cover slips. The 24 well plate can be reused **ONE TIME ONLY.**

___12. From the DLS data, select the best buffer condition giving the smallest radius (<5 nm), lowest polydispersity (<30%) and highest intensity when doing DLS measurement. After the protein has been placed in the new buffer, concentrate it and set up crystallization trials.

If condition is not optimal, proceed to use additives.

*** Additive Screening (Common additives used are 5% glycerol, 10% glycerol, 25, 50, and 100 mM NaCl, 0.1%, 1% octylglucoside, 10 mM beta-mercaptoethanol, 5 mM dithiothreitol, 2 mM CHAPS (final concentration))**

___A. Use only enough protein to test for the additives. Calculate how much protein you would need: # of additives x protein (2 mg/ml) x 20 µl. Estimate some loss during concentration and use this amount to exchange your protein into the best buffer condition found above (Step 12) at a concentration of **50 mM**. Use a concentrator (Apollo, Ultrafree, etc.) to concentrate your protein: (a) add the selected buffer to your protein (1:5 dilution, invert gently), concentrate down; (b) add more buffer (1:5 dilution) and concentrate again; (c) repeat step (b) one more time. You could alternatively dialyse the protein against the selected buffer and then concentrate it to 2 mg/ml.

___B. Based on the protein's properties, decide which additives are going to be tested with the selected buffer (e. g., if it has cysteines you may want to use beta-mercaptoethanol or dithiothreitol).

___C. You want the total volume of the sample to be 15-20 µl and your protein concentration to be 1-2 mg/ml. Place 15-20 µl of protein (1-2 mg/ml) in 0.5 ml microcentrifuge tubes and add stock additives to each of the tubes to desired concentration. Mix gently by pipetting up and down.

___D. Let these samples incubate for 30-60 minutes at room temperature, but preferably overnight.

___E. Spin the samples in a microfuge for 5 min and perform DLS on each sample, compare to the results from Step 12, and determine the optimal condition.

___F. The optimal buffer with the best additive is then used to exchange the protein using a concentrator as indicated in Step A. Crystallization trials are then performed.

Buffers are prepared as 1 M Stock Solutions except for Buffer # 3. Working solutions are used as 0.1 M solutions. Buffers are kept at 4 C except for Buffer # 3.

Buffers for Optimum Solubility Screen

	Buffer(1M)	pH	Vendor	Recipe for 50mL, 1 M Stock Solutions
1	Glycine	3	Fluka	3.75 g Glycine
2	Citric Acid	3.2	Fluka	9.62 g citric acid
3	**PIPPS**	**3.7**	**Calbiochem**	**8.26 g PIPPS=0.5 M, keep at room temperature.**
4	Citric Acid	4	Fluka	9.62 g citric acid
5	Sodium acetate	4.5	Fluka	4.10 g sodium acetate
6	Na K phosphate	5	Fluka	6.80 g H_2KO_4P + 6.0 g H_2NaO_4P
7	Sodium citrate	5.5	Fluka	14.7 g sodium citrate
8	Na K phosphate	6	Fluka	6.80 g H_2KO_4P + 6.0 g H_2NaO_4P
9	Bis Tris	6	Fluka	10.46 g Bis Tris
10	MES	6.5	Fluka	10.66 g MES
11	ADA	6.5	Fluka	9.5 g ADA + ~5 ml 10M NaOH + H_2O up to 50 ml
12	Bis Tris Propane	6.5	Fluka	14.12 g Bis Tris Propane
13	Cacodylate	6.5	Fluka	10.7 g Na Cacodylate
14	Ammonium acetate	7	Fluka	3.85 g Ammonium acetate
15	MOPS	7	Sigma	10.46 g MOPS
16	Na K phosphate	7	Fluka	6.80 g H_2KO_4P + 6.0 g H_2NaO_4P
17	HEPES	7.5	Fluka	13.02 g HEPES
18	TRIS	7.5	Sigma	6.055 g TRIS Base
19	EPPS	8	Fluka	12.62 g EPPS
20	Imidazole	8	Research Organics	33.3 ml 1.5M stock Imidazole + H_2O up to 50 ml
21	TRIS	8.5	Sigma	6.055 g TRIS Base
22	CHES	9	Fluka	10.36 g CHES
23	CHES	9.5	Fluka	10.36 g CHES
24	CAPS	10	Fluka	11.06 g CAPS

List of Additives (final concentration):

10 mM BME
2 mM CHAPS
5mM DTT
5% glycerol
10% glycerol
25 mM NaCl
50 mM NaCl
100 mM NaCl
0.1% Octylglucoside
1.0% Octylglucoside