User Guide

JBS Solubility Kit

Optimization of protein buffer composition prior to crystallizations trials

JENA BIOSCIENCE

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Introduction

Since the highly complex properties of proteins are dependent on their environment, buffer solutions play an important role, i.e. influencing the solubility and the aggregation behaviour of the protein sample. Studies have shown that aggregation of the protein may inhibit nucleation and crystal growth.
Therefore, the **JBS Solubility Kit** has been designed to investigate protein samples towards their homogeneity and monodispersity in dependence of the buffer solution, employing hanging-drop vapour diffusion experiments combined with dynamic light scattering.

The JBS Solubility Kit

The **JBS Solubility Kit** is a pre-crystallization screen to improve the composition of the initial protein buffer solution prior to performing crystallization set-ups [1] .

The **JBS Solubility Kit** comprises two individual kits for successive use:

A: Buffer Kit
24 buffer solutions at different pH values, 100 mM, supplied in 10 ml volumes

No.	Buffer	pH
1	Glycine	3.0
2	Citric Acid	3.2
3	PIPPS	3.7
4	Citric Acid	4.0
5	Sodium Acetate	4.5
6	Sodium / Potassium Phosphate	5.0
7	Sodium Citrate	5.5
8	Sodium / Potassium Phosphate	6.0
9	Bis-Tris	6.0
10	MES	6.2
11	ADA	6.5
12	Bis-Tris Propane	6.5
13	Ammonium Acetate	7.0
14	MOPS	7.0
15	Sodium / Potassium Phosphate	7.0
16	HEPES	7.5
17	Tris	7.5
18	EPPS	8.0
19	Imidazole	8.0
20	Bicine	8.5
21	Tris	8.5
22	CHES	9.0
23	CHES	9.5
24	CAPS	10.0

B: Additive Kit
14 Additives solutions, supplied in 250 µl volumes, ready to use, concentration adjusted

No.	Additive	Concentrated Stock Solution
1	Sodium Chloride	80 mM
2	Sodium Chloride	200 mM
3	Sodium Chloride	400 mM
4	Glycerol	20%
5	Glycerol	40%
6	CHAPS	8 mM
7	Octyl Glucopyranoside	0.4 %
8	Octyl Glucopyranoside	4%
9	Dodecyl Maltoside	0.4 %
10	Dodecyl Maltoside	4%
11	BME	40 mM
12	DTT	4 mM
13	DTT	20 mM
14	TCEP	120 mM

Instructions

The screening for a suitable buffer solution is performed in three successive steps:

1. ***Hanging-drop experiment***
2. ***Dynamic light-scattering analysis (DLS)***
3. ***Additive Screen***

Hanging-drop experiment

In the hanging-drop experiment (see Fig. 1), a drop composed of a mixture of protein and buffer solution is equilibrated against a larger reservoir of buffer solution. For this experiment 24 different buffers at different pH values are used, whereby one can visualize the dependence of protein aggregation on the buffer solution in a pH range of 3.0–10.

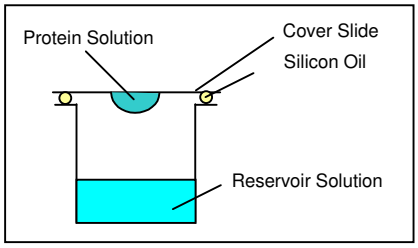

Figure 1. The hanging-drop method

1. Use a 24 well crystallization plate. Apply silicone grease evenly to the upper surface of the circular edges around the individual reservoirs.
2. Pipette 0.5 ml of each buffer solution of Buffer Kit (**A**) into the individual reservoir wells.
3. Concentrate your protein solution as high as possible.
4. Pipette 1 µl protein solution and 1 µl reservoir solution onto a round glass cover slip. First apply the drop of protein solution to the center of the cover slide. Subsequently, add 1 µl of the reservoir solution to each drop. Mix carefully.
5. Mount the cover slides with tweezers. Each cover slide is inverted and gently placed over the corresponding reservoir so that the hanging drop is positioned over the center of the reservoir.

Store the prepared crystallization plate in a suitable area at room temperature. Over the course of time, water from the drop diffuses as water vapor into the reservoir solution. This raises the concentration of the protein in the drop. After an incubation time of 24 hours, the drops are investigated under a light microscope. At this stage, different degrees of precipitation depending on the employed buffer may be noticed.

Dynamic light-scattering analysis

This method provides information on the degree of aggregation of the protein within the buffer solution.
The drops which remained clear indicate sufficient solubility of the protein in the buffer and will be further investigated using dynamic light scattering.

1. Carefully flip the cover slides back and pay special attention that the slides do not break.
2. Dilute the clear drops with 18 µl of reservoir solution into a microfuge tube.

   (***NB:*** It is important to note that the minimal protein concentration for this experiment should be **not** less than 1 mg/ml. This implies that the initial protein concentration should be at least 20 mg/ml. If this cannot be maintained, larger drops are recommended for the hanging-drop experiment.)
3. Spin down the sample at high speed for 10 min before starting the DSL experiment.
4. Follow the instructions of your Dynamic light scattering instrument.

If a narrow, monomodal size distribution and small or negligible polydispersity (< 25 %) is observed the protein sample can be transferred directly into the respective buffer solution at a concentration of 20 mM and used for crystallization screening.

Additive Screen

If no buffer of **Kit A** yielded a monodisperse protein solution, the results can be improved by adding the additives from **Kit B**.

1. Select the buffer which yielded the best DLS reading.
2. Transfer the protein into this buffer. A buffer concentration of 50 mM is recommended. The protein concentration should not be less than 2-3 mg/ml.
3. Pipette 15 µl of protein sample and 5 µl of the individual additive into a microfuge tube and mix thoroughly.
4. Incubate this solution for 2 hours at room temperature.
5. Spin down the sample for 10 min and perform an additional DLS analysis.
6. Select the best condition and dialyze the protein into the respective buffer with the corresponding additive.
7. Concentrate the protein solution to approximately 10 mg/ml and use this for crystallization screening.

For successful crystallization screening, **Jena Bioscience** offers a large range of products**,** i.e. **JBScreen Classic, JBScreen Cryo** or **JBScreen Basic.**

References

[1] 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.