IFTA AG
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JBScreen Solubility HTS

Cat. No.	Amount
CO-311	95 solutions, 1.7 ml each

For *in vitro* use only
Store at 4°C

Introduction

Characterization of proteins by either structural, biochemical or biophysical means requires large amounts of pure protein. In order to obtain enough material, the protein is purified by a number of chromatographic steps, with the whole procedure often taking 2-3 days. Once the protein is purified it needs to be stored: frequently frozen at -80°C, and sometimes at 4°C. For purification and storage a buffer composition is chosen to keep the protein in solution. However, proteins differ greatly in their properties and hence suitable buffer conditions change significantly between different proteins. Using sub-optimal buffer conditions leads to protein aggregation and loss of protein during purification and storage. It can also dramatically decrease the success rate of crystallization trials and can give incorrect results during biochemical and biophysical analysis. It is therefore very important to find optimal buffer conditions for each individual protein or protein complex. However, the search for optimal conditions is tedious and therefore often ignored.

JBScreen Solubility HTS represents a quick and inexpensive way to find suitable buffer conditions to purify and store the proteins in. The screen has been tested successfully on a wide variety of protein by different researchers at the LMB/MRC Cambridge.

Previous Work & Novelty

Previously, two protein solubility screens have been described by Lepre [1] and Jancarik [2], the latter resulted in the JBS Solubility Kit (Cat.# CO-310). Both screens are based on a manual setup and therefore require a considerable amount of time and protein. In addition, both screens use equal ratio of protein and test condition, thereby making the effect of the buffer components in which the protein is stored more dominant. Furthermore, due to a smaller number of conditions tested in the first round, multiple rounds of testing may be required to achieve the best condition. JBScreen Solubility HTS comes premade in a deep well block. Standard crystallization robots quickly setup the assay (< 5 min) and very small amounts of protein are used (10 µl @ 5-10 mg/ml). Also, the

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protein and buffer conditions are dispensed in a ratio of 1:3, thereby minimizing the effect of any buffer components in which the protein is stored. Finally, because, the screen tests for buffer, pH, salt and glycerol at the same time, there is no need for follow up screens.

Description of JBScreen Solubilty HTS

The screen contains 24 conditions that differ by buffer chemicals and by pH. These 24 conditions are presented 4 times in the screen: with the buffer components alone, with 150 mM NaCl, with 5% glycerol, and with 150 mM NaCl and 5% glycerol.

An empty well (# H12) is added to compare the current protein buffer with the buffer conditions of the screen. In addition, a positive control for precipitation (20% acetonitrile, well# A1) is included in the screen.

Please check the data sheet for exact formula compositions.

Experimental Protocol

The screen is set up as sitting drop experiment using a standard sitting drop crystallization plate.

1. Add ~ 70 µl of each condition to the corresponding reservoir well.
2. Add the same volume of your current protein buffer to the reservoir well H12.
3. Mix 100 nl of protein solution (1-40 mg/ml) into a 300 nl drop of well solution.
4. Seal the plate with a transparent film and incubate @ room temperature or 4°C.

Protein precipitation is visible using a standard light microscope after ~ 1 hour at high protein concentrations (> 5 mg/ml) or 12 hours at low protein concentrations (< 5 mg/ml).

For optimal viewing of the drops, adjust the angle of the incident light such that the most of the drop is dark (Figure 1). When light is scattered of the precipitated protein it will light up as clear white flakes in a dark field (Figure 2).

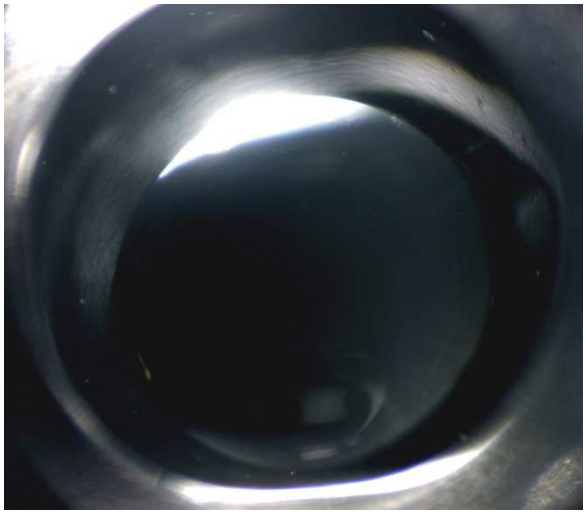

Figure 1: Clear protein droplet indicates suitable buffer condition

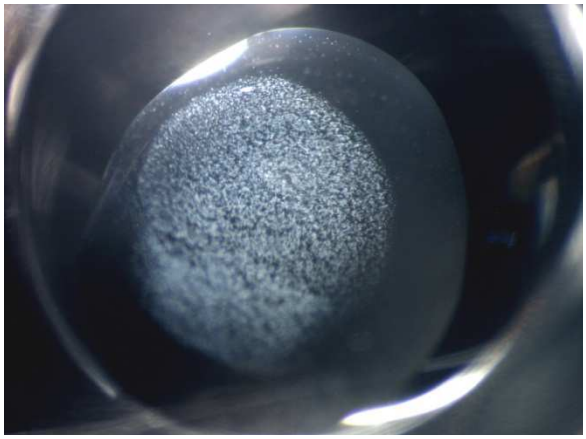

Figure 2: Protein droplet with protein precipitation

References

[1] Lepre *et al.* (1998) Microdrop screening: a rapid method to optimize solvent conditions for NMR spectroscopy of proteins. *J Biomol NMR* **12(4)**:493

[2] 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