Center for Eukaryotic Structural Genomics

Technology Dissemination Report

CESG Tech Report No.	030
Title	Detergent Solubilization Screening for Membrane Proteins Expressed in *E.coli*
Research Unit	Protein Production (Large Scale Protein Production)
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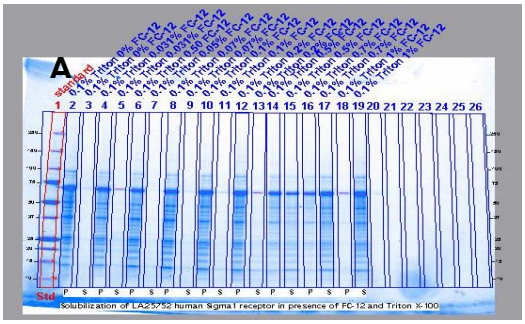
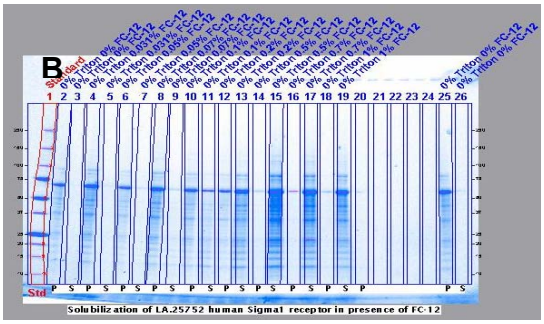
FC-12 →	0%	0.031%	0.05%	0.07%	0.10%	0.20%	0.50%	0.70%	1%
Triton X-100 ↓	1	2	3	4	5	6	7	8	9
A 0.01%	0% FC-12 0.01% Triton X-100	0.031% FC-12 0.01% Triton X-100	0.05% FC-12 0.01% Triton X-100	0.05% FC-12 0.01% Triton X-100	0.1% FC-12 0.01% Triton X-100	0.2% FC-12 0.01% Triton X-100	0.5% FC-12 0.01% Triton X-100	0.7% FC-12 0.01% Triton X-100	1% FC-12 0.01% Triton X-100
B 0.05%	0% FC-12 0.05% Triton X-100	0.031% FC-12 0.05% Triton X-100	0.05% FC-12 0.05% Triton X-100	0.05% FC-12 0.05% Triton X-100	0.1% FC-12 0.05% Triton X-100	0.2% FC-12 0.05% Triton X-100	0.5% FC-12 0.05% Triton X-100	0.7% FC-12 0.05% Triton X-100	1% FC-12 0.05% Triton X-100
C 0.1%	0% FC-12 0.1% Triton X-100	0.031% FC-12 0.1% Triton X-100	0.05% FC-12 0.1% Triton X-100	0.05% FC-12 0.1% Triton X-100	0.1% FC-12 0.1% Triton X-100	0.2% FC-12 0.1% Triton X-100	0.5% FC-12 0.1% Triton X-100	0.7% FC-12 0.1% Triton X-100	1% FC-12 0.1% Triton X-100
D 0.5%	0% FC-12 0.5% Triton X-100	0.031% FC-12 0.5% Triton X-100	0.05% FC-12 0.5% Triton X-100	0.05% FC-12 0.5% Triton X-100	0.1% FC-12 0.5% Triton X-100	0.2% FC-12 0.5% Triton X-100	0.5% FC-12 0.5% Triton X-100	0.7% FC-12 0.5% Triton X-100	1% FC-12 0.5% Triton X-100
E									
F									
G 0%	0% FC-12 0% Triton X-100	0.031% FC-12 0% Triton X-100	0.05% FC-12 0% Triton X-100	0.05% FC-12 0% Triton X-100	0.1% FC-12 0% Triton X-100	0.2% FC-12 0% Triton X-100	0.5% FC-12 0% Triton X-100	0.7% FC-12 0% Triton X-100	1% FC-12 0% Triton X-100

Solubilization array for MBP-human sigma1 receptor. The concentrations of detergents that solubilize over 90% of protein are marked in yellow.

Solubilization screening of MBP-sigma1 receptor (LA.25752) in presence of (**A**) Triton X-100 and fos-choline-12 (FC-12) and (**B**) fos-choline-12 (FC-12). S- soluble fraction, P- insoluble fraction (pellet).





Summary

CESG is developing new purification methods for membrane proteins expressed in *Escherichia coli* (*E. coli*), that is based on a high-throughput detergent solubilization screen and IMAC protein purification. In case there is no previous research done on a particular membrane protein, this screening procedure starts with wide variety of detergents across a range of concentrations.

Here we present an approach to solubilize a human sigma1 receptor expressed as N-terminal MBP fusion (with signal sequence that directs it to the periplasm – plasmid pVP87K). This protein has been extensively studied but the structure has yet to be solved [1]. To perform this screen in a 96 well plate, it is sufficient to start with 0.1 g of wet cell paste. After breaking the cells by sonication, the lysate is centrifuged at 16,000g to separate the insoluble and soluble fractions. The pellet (insoluble fraction) is then re-suspended and used in the high-throughput detergent solubilization screens. The testing is performed at 4ºC overnight, and then the solubilized fraction is separated by centrifugation. We employ the Bio-Rad stain free imager to estimate the efficiency of solubilization.

This method can be semi- or fully automated, and is serving as a precursor for automated purification using the Maxwell 16 system.

[1] Ramachandran, S., Lu, H., Prabhu, U. and. Ruoho,A.E. (2007) Purification and characterization of the guinea pig sigma-1 receptor functionally expressed in *Escherichia coli*. *Protein Expr Purif.* 51(2): 283-92'

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