Product Manual

Rapid GST Inclusion Body Solubilization and Renaturation Kit

Catalog Number

AKR-110

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Bacteria are widely used for His or GST tagged recombinant protein expression. GST fusion proteins in soluble form are purified from bacterial lysates by affinity chromatography using immobilized glutathione. However, recombinant proteins expressed in bacteria often form inclusion bodies, especially when they are expressed at high levels. It is not known exactly how they are formed, but it is thought that the protein within the inclusion body is partially or incorrectly folded. Once these inclusion bodies are formed, it is very difficult to solubilize them in a native, active conformation. The Rapid GST Inclusion Body Solubilization and Renaturation Kit is designed to retrieve expressed GST fusion protein in soluble form after lysis and extraction procedures.

The detergent solubilization and neutralization reagents contained in the kit provides the most effective means for solubilizing and renaturing aggregated proteins without lengthy dialysis steps. The solubilization and neutralization steps only take 2 hrs (see Figure 1). The kit provides enough reagents for solubilizing and renaturing up to 5-10 liters of bacterial culture.

The Cell Biolabs Rapid GST Inclusion Body Solubilization and Renaturation kit contains a proprietary detergent formulation that provides several advantages over conventional GuHCl or Urea solubilization and refolding method:

- Designed specifically for solubilizing and renaturing GST inclusion bodies
- Time saving: without lengthy dialysis or dilution step
- No pH variation and Redox Pair involved, easy to use

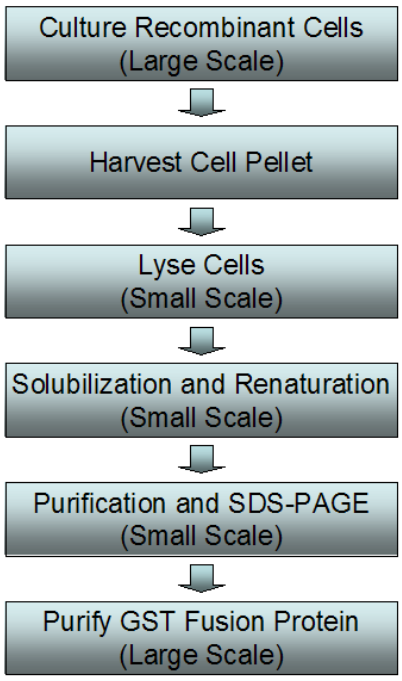

Figure 1 – GST Inclusion Bodies Solubilization and Renaturation Flow Chart

Kit Components

1. 10X STE Buffer (Part No. 411001): One bottle - 120 mL of 500 mM Tris, pH 7.5, 1.5 M NaCl, 10 mM EDTA

2. Detergent Solubilization Solution (Part No. 411002): One bottle - 60 mL
3. Detergent Neutralization Solution (Part No. 411003): One bottle - 60 mL

Materials Not Supplied

1. Lysozyme
2. Proteinase Inhibitor Cocktail
3. Glutathione Agarose Bead Slurry
4. PBS containing 1% Triton X-100
5. Reduced Glutathione
6. Heating Block

Storage

Store all kit components at room temperature.

Preparation of Reagents

- 1X STE Extraction Buffer: freshly add 1 mM of DTT, 0.2 mg/mL of Lysozyme and proteinase inhibitor cocktail when diluting 10X STE Buffer to 1X STE Extraction Buffer with dH_2O. Keep the solution on ice.
- Diluted Detergent Solubilization Solution: according to Table 1, prepare a serial of two-fold dilution of Detergent Solubilization Solution with 1X STE Extraction Buffer.

Tubes	Detergent Solubilization Solution (µL)	1X STE Extraction Buffer (µL)
1	300	0
2	150 of Tube #1	150
3	150 of Tube #2	150
4	150 of Tube #3	150
5	0	150

Table 1. Dilution of Detergent Solubilization Solution

Assay Protocol

I. Induction of recombinant GST fusion protein expression in *E. coli* culture.

Induction conditions, such as IPTG concentration, culture temperature and time, should be decided by the user.

II. Bacterial cell lysis, inclusion body solubilization and renaturation

1. Pellet 200 mL of *E. Coli* culture by spinning 10 minutes at 5000 g at 4ºC.
2. Resuspend cell pellet in 10 to 20 mL of cold 1X STE Extraction Buffer. Break cells by brief pulses of sonication on ice until the sample is no longer viscous.
3. Transfer 0.9 mL of cell lysate/inclusion body mixture to a tube and add 100 µL of diluted Detergent Solubilization Solution including undiluted Detergent Solubilization Solution and 1X STE Extraction Buffer as a blank (see Table 1) . Incubate on ice for one hour. Mix by inversion occasionally.
4. Spin 15 minutes at 12000 g, transfer 0.9 mL of supernatant to another tube.

5. Add 100 µL of Detergent Neutralization Solution. Incubate on ice for one hour. Mix by inversion occasionally. Save 50 µL for SDS-PAGE analysis.

III. GST Purification and SDS-PAGE

1. Add 50 µL of Gluthione Agarose beads (50% slurry) to the 1 mL cell extract containing renatured GST fusion protein.
2. Incubate 1-2 hr at room temperature or overnight at 4ºC. Mix by inversion.
3. Wash beads three times with 1X PBS containing 1% Triton X-100.
4. Carefully aspirate all supernatant and add 25 µL of 2X SDS-PAGE Sample Buffer directly to the washed beads. Vortex and heat 5 minutes on a heating block.
5. Determine the optimal detergent amount for solubilizing and renaturing GST inclusion body by running a SDS-PAGE.

IV. Fusion Protein Purification

1. Purify in large scale using the optimal detergent amount as defined above.
2. To ensure maximal recovery of renatured GST fusion protein, we recommend overnight incubation of cell extract with GS-beads at 4ºC.

Example of Results

The following figures demonstrate typical results with the Rapid GST Solubilization and Renaturation Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

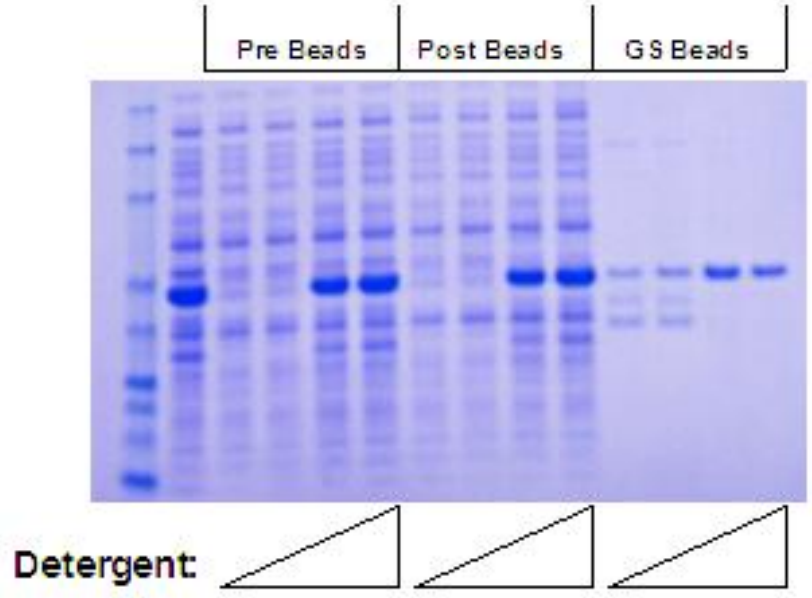

Figure 2 – Solubilization and Renaturation of GST-RTK fusion protein. GST-RTK expression was induced with 1 mM IPTG at 37ºC for 4 hrs. Cell pellet was lysed, and inclusion body was solubilized and renatured under different amounts of detergent solubilization solution according to the assay protocol. Lane 1: MW STD; Lane 2: Whole E.Coli lysate; Lane: 3, 7, 11: No detergent; Lane 4, 8, 12: 32-fold dilution; Lane 5, 9, 13: 8-fold dilution; Lane 6, 10, 14: 2-fold dilution.

References

1. Hamel,V., et al. (2017). Identification of Chlamydomonas Central Core Centriolar Proteins Reveals a Role for Human WDR90 in Ciliogenesis. Curr Biol. **27**(16):2486-2498.e6. doi: 10.1016/j.cub.2017.07.011.
2. Shin SI, GST Gene Fusion System Handbook, Amersham Biosciences, Code No. 18-1157-58.

Recent Product Citations

1. Gao, Q. et al. (2019). Purification of insoluble GST-fused and GST-cleaved Cav1.2 channel fragment by denaturation and renaturation. *Protein Expr Purif*. **160**:7-10. doi: 10.1016/j.pep.2019.03.013.
2. Campion, C.G. et al. (2018). COMMD5/HCaRG Hooks Endosomes on Cytoskeleton and Coordinates EGFR Trafficking. *Cell Rep*. **24**(3):670-684.e7. doi: 10.1016/j.celrep.2018.06.056.
3. Wang, H. et al. (2017). Truncated protein tyrosine phosphatase receptor type O suppresses AKT signaling through IQ motif containing GTPase activating protein 1 and confers sensitivity to bortezomib in multiple myeloma. *Oncotarget*. **8**(69):113858-113873. doi: 10.18632/oncotarget.23017.
4. Dixon, J. E. et al. (2016). Highly efficient delivery of functional cargoes by the synergistic effect of GAG binding motifs and cell-penetrating peptides. *Proc Natl Acad Sci U S A.* **113**:E291-E299.
5. Matsuoka, T. et al. (2014). Expression and characterization of honeybee, Apis mellifera, larva chymotrypsin-like protease. *Apidologie*. doi:10.1007/s13592-014-0313-2.
6. Keller, D. et al. (2014). Mechanisms of HsSAS-6 assembly promoting centriole formation in human cells. *J. Cell Biol.* **204**:697-712.
7. Lalani, S. et al. (2013). MCTP2 is a dosage-sensitive gene required for cardiac outflow tract development. *Hum. Mol. Genet*. 10.1093/hmg/ddt283.
8. Sabbah, M. et al. (2011). CCN5, A novel transcriptional repressor of transforming growth factor-ß signaling pathway. *Mol. Cell. Biol*. 10.1128/MCB.01316-10.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com