Datasheet for ABIN2344796

Rapid GST Inclusion Body Solubilization and Renaturation Kit

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Overview

Quantity:	1 kit
Reactivity:	Bacteria
Application:	Purification (Purif)

Product Details

Purpose:	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.
Characteristics:	The 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 2
Components:	1. 10X STE Buffer : One bottle - 120 mL of 500 mM Tris, pH 7.5, 1.5 M NaCl, 10 mM EDTA 2. Detergent Solubilization Solution : One bottle - 60 mL 3. Detergent Neutralization Solution : One bottle - 60 mL
Material not included:	1. Lysozyme 2. Proteinase Inhibitor Cocktail 3. Glutathione Agarose Bead Slurry

Product Details

4. PBS containing 1 % Triton X-100
5. Reduced Glutathione
6. Heating Block

Target Details

Background:	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.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Quickly solubilize and renature GST inclusion bodies • No lengthy dialysis or dilution steps • No pH variation or redox pair involved
Reagent Preparation:	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 dH2O. 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. 1X STE Detergent Solubilization Extraction Tubes Solution (µL) 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 3
Assay Procedure:	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 4 °C. 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.

Application Details

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 4 °C. 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 4 °C. 4

Restrictions:	For Research Use only

Handling

Storage:	RT
Storage Comment:	Store all kit components at room temperature.

Publications

Product cited in:

Dixon, Osman, Morris, Markides, Rotherham, Bayoussef, El Haj, Denning, Shakesheff: "Highly efficient delivery of functional cargoes by the synergistic effect of GAG binding motifs and cell-penetrating peptides." in: **Proceedings of the National Academy of Sciences of the United States of America**, Vol. 113, Issue 3, pp. E291-9, (2016) (PubMed).

Keller, Orpinell, Olivier, Wachsmuth, Mahen, Wyss, Hachet, Ellenberg, Manley, Gönczy: " Mechanisms of HsSAS-6 assembly promoting centriole formation in human cells." in: **The Journal of cell biology**, Vol. 204, Issue 5, pp. 697-712, (2014) (PubMed).

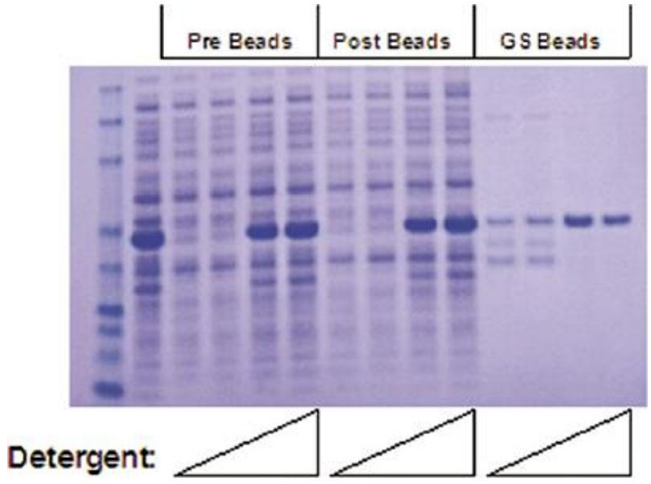
Lalani, Ware, Wang, Zapata, Tian, Franco, Jiang, Bucasas, Scott, Campeau, Hanchard, Umaña, Cast, Patel, Cheung, McBride, Bray, Craig Chinault, Boggs, Huang, Baker, Hamilton, Towbin, Jefferies et al.: "MCTP2 is a dosage-sensitive gene required for cardiac outflow tract

development. ..." in: **Human molecular genetics**, Vol. 22, Issue 21, pp. 4339-48, (2013) (PubMed).

Sabbah, Prunier, Ferrand, Megalophonos, Lambein, De Wever, Nazaret, Lachuer, Dumont, Redeuilh: "CCN5, a novel transcriptional repressor of the transforming growth factor ? signaling pathway." in: **Molecular and cellular biology**, Vol. 31, Issue 7, pp. 1459-69, (2011) (PubMed).

Kawabata, Sugiyama, Kuwamura, Odaka, Satoh: "Simultaneous determination of tegafur and 5-fluorouracil in serum by GLC using nitrogen-sensitive detection." in: **Journal of pharmaceutical sciences**, Vol. 72, Issue 10, pp. 1162-5, (1984) (PubMed).

Validation report #104253 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)



SDS-PAGE

Image 1. Solubilization and Renaturation of GST-RTK fusion protein. GST-RTK expression was induced with 1 mM IPTG at 37ºC for 4 hours. The cell pellet was lysed, and inclusion bodies were solubilized and renatured under different amounts of detergent solubilization solution according to the assay protocol. Lane 1: MW Standard; Lane 2: Whole E. coli lysate; Lanes 3, 7, 11: No detergent; Lanes 4, 8, 12: 32-fold dilution; Lanes 5, 9, 13: 8-fold dilution; Lanes 6, 10, 14: 2-fold dilution.