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A Geno Technology, Inc. (USA) brand name

IBS™ Buffer Kit

For Inclusion Body Solubilization

(Cat. # 786-183, 786-183S)

INTRODUCTION

Bacterial expression of recombinant proteins is a commonly used technique. A major problem with bacterial expression is that non-bacterial and over-expressed proteins form aggregates of insoluble proteins, known as inclusion bodies. The reasons for inclusion body formation are poorly understood, however it is hypothesized that the exposed hydrophobic domains of partially or incorrectly folded proteins aggregate together to form the large insoluble aggregates. The large, aggregate nature of inclusion bodies allows for them to be separated by centrifugation.

Once separated from the soluble proteins, the large aggregates are themselves difficult to solubilize. The IBS™ Buffer kit contains a proprietary buffer that gently solubilizes the inclusion bodies.

The chemical composition of the supplied IBS™ Buffer and denaturant are compatible with widely used protein folding protocols. IBS™ Buffer is also compatible for protein estimation with our NI™ protein assay (Cat. # 786-005) and running SDS-PAGE analysis using PAGE-Perfect™ kit (Cat# 786-123).

ITEM(S) SUPPLIED

Description	Cat. # 786-183	Cat. # 786-183S
IBS™ Buffer	100ml	10ml
DTT [1M in 1ml]*	1 Vial	-

* See Preparation before use

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival store at 4°C and is stable for 1 year.

ADDITIONAL ITEMS NEEDED

Centrifuge and test tubes

PREPARATION BEFORE USE

Add 1ml de-ionized water to the DTT vial. Vortex until dissolved to achieve a 1M DTT solution. Store at -20°C.

ISOLATION OF INCLUSION BODIES

Inclusion bodies are easily isolated following bacterial lysis by centrifugation. For inclusion bodies isolation, we recommend using Bacterial-PE LB™ (Cat. # 786-176). Briefly, incubate the bacterial cells in Bacterial-PE LB™ and lysozyme until lysis is complete. At the end of incubation, centrifuge the lysate at 30,000xg for 30 minutes at 4°C. Collect the pellet and wash twice with 1 in 10 dilution of Bacterial-PE LB™. Collect the inclusion bodies for solubilization and folding.

INCLUSION BODY SOLUBILIZATION PROTOCOL

1. Determine the wet weight of inclusion bodies and resuspend each 100mg inclusion bodies in 1ml IBS™ Buffer.
2. If disulfide bonds are involved in folding, add 20µl/ml 1M DTT to the suspended inclusion bodies to give a final concentration of 20mM DTT.
3. Suspend the inclusion bodies by vortexing or pipetting up and down until a homogeneous suspension is achieved.
4. Shake the suspension for 1 hour at 4°C for complete solubilization.
5. Remove insoluble material that may affect downstream processing by centrifugation at 30,000xg for 15 minutes.
6. Collect the supernatant for further analysis.

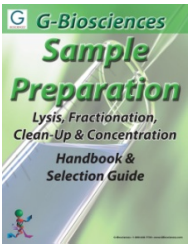
PROTEIN FOLDING

The presence of a denaturant results in unfolded proteins, so to obtain an active protein the proteins must be refolded. Every protein has unique folding properties and is therefore difficult to suggest a universal folding protocol.

We recommend determining the optimal folding protocol using our PROTEIN-Foldase™ Kit (Cat. # 786-185), which is designed to simplify the optimization of a protein folding protocol.

RELATED PRODUCTS

Download our Sample Preparation Handbook.



http://info.gbiosciences.com/complete-protein-sample-preparation-handbook/

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