

HiPurA® Bacterial Protein Extraction Reagent (Ionic)

<u>Product Name</u>	<u>Product Code</u>	<u>Kit Packing</u>
HiPurA® Bacterial Protein Extraction Reagent (Ionic)	ML223-100ML	100ml
	ML223-250ML	250ml
	ML223-500ML	500ml

Intended Use:

Recommended for efficient extraction of total soluble protein in non-denatured state from Bacterial cells.

Introduction:

Bacteria are increasingly used for the production of industrially relevant proteins. The ease with which bacteria accept and express new genes allows for the use of these cells in multitude of applications particularly remarkable is the impact of recombinant proteins on biopharmaceutical applications. The engineering of such systems could represent a promising way to the cost effective production of high quality protein versions that biotechnology and biomedical industries are steadily demanding. Successful applications of protein based techniques relies on effective cell lysis, recovery and characterization of proteins.

Efficient extraction of these proteins is a great challenge for numerous downstream proteomic analysis. There are several methods available for extraction of enriched proteins from different bacterial cells such as shearing force, treatment with low ionic salt (salting out) and rapid changes in pressure, sonication, French press, homogenization, manual grinding or using blenders. These methods for protein extraction are usually cumbersome due to heavy equipment, reduced yields as the sample is processed through several steps and many times not able to maintain protein stability, integrity and functionality. In recent years, detergent based lysis methods have become the norm. Here, we have designed a detergent based formulation for extracting proteins from bacterial cells in a simple and gentle manner.

Description:

HiPurA® Bacterial protein extraction reagent (ionic) is based on a proprietary combination of organic buffering agents and mild ionic detergents to enhance extraction of proteins and maintain stability of biological activities of the proteins. This reagent effectively extracts soluble proteins from several common bacterial host strains and is especially suitable for the protease-defective expression host BL21 strains. This reagent can extract proteins from recently prepared cells as well as frozen cells.

Applications:

HiPurA[®] Bacterial Protein Extraction Reagent (ionic) is a ready-to-use reagent which ensure good protein recovery, while maintaining the biological activity of the proteins. The solubilized proteins are suitable for enzyme assays, electrophoresis, folding studies, protein purification, chromatographic studies and many other downstream applications.

Also, the reagent is compatible with standard protein assays such as BCA Protein Assay and Coomassie – Blue, X-press Blue and silver staining.

Composition:

Bacterial Protein Extraction Reagent (ionic) contains a proprietary ionic detergent in Tris-Cl (pH 8.0) and EDTA buffer system.

Properties:

Appearance/ Color/ Clarity : Colorless clear solution and free of particles.

DNase & RNase : None detected

pH : 7.9 - 8.1

Suitability Test : This product has been tested and is suitable for use in extracting proteins from bacteria.

Storage Conditions:

HiPurA[®] Bacterial Protein Extraction Reagent (ionic) can be stored at room temperature (15-25°C). Under recommended condition, the reagent is stable for 6 months.

Material needed but not provided:

- Protease Inhibitor Cocktail (Product Code: ML051)
- Bacterial cells.
- 1X PBS Solution (Product Code: ML116) / 10X PBS Solution (Product Code: ML023)
- Centrifuge Tubes
- Centrifuge

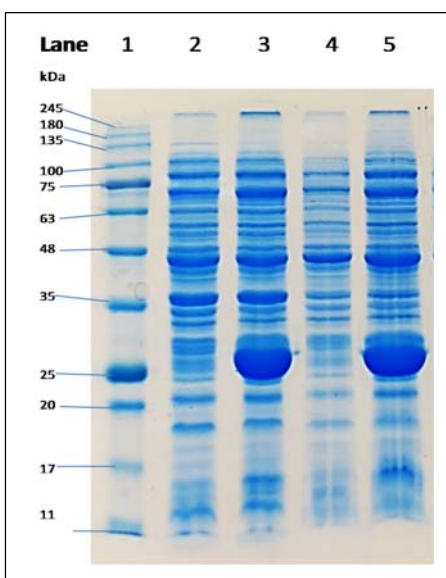
Protocol:

Note: The reagent is filter sterilized and should be open under aseptic conditions.

1. Centrifuge bacterial cells (O.D₆₀₀ – 1.5-3.0) at 5000 rpm for 10 minutes at RT. Discard the supernatant (Media).
Optional: Wash the cell pellet with 1XPBS. Centrifuge at 5000 rpm for 5-10 minutes. Discard the supernatant
2. Calculate the weight of cell pellet as follows:
Weight of cell pellet = Weight of tube with cell pellet - Weight of empty tube.

3. For each 1gm of wet cell pellet add 4ml of HiPurA® Bacterial Protein Extraction Reagent (ionic).
(**Note:** If desired, add Protease Inhibitor cocktail (ML051) to extraction buffers just before use. Take out require amount of buffer in collection tube and then add ML051 (100X to 1X)).
4. Homogenize the cell pellet with reagent by pipetting up and down very gently. Incubate for 10-15 minutes at RT.
5. Centrifuge the lysate at 13,000 rpm for 5 -10 minutes.
6. Collect the supernatant and proceed for further downstream processing or store at -20°C.

Data:



Well No.	Sample
1	Prestained protein ladder
2	Protein extracted from uninduced bacterial cell culture using competitor reagent
3	Protein extracted from IPTG induced bacterial cell culture using competitor reagent
4	Protein extracted from uninduced bacterial cell culture using ML223
5	Protein extracted from IPTG induced bacterial cell culture using ML223

Fig.1: SDS-PAGE gel image representing protein extracted from E.coli host cells (BL21PGEX4T2) using bacterial protein extraction reagents.

Troubleshooting:

Problem	Cause	Solution
Low Protein Yield	Low protein expression	Optimize transfection procedure
	Insufficient amount of reagent or less incubation time for cell lysis	Add more reagent and increase incubation time and shake more vigorously during incubation
Degraded Protein	Protease contamination	Add Protease Inhibitor cocktail to the reagent prior to use

Warning and Precautions

Not for Medicinal Use. Read the SDS carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Performance and Evaluation

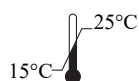
Performance of the solution is expected when the solution is stored at recommended temperature and within the expiry period.

Safety Information

The HiPurA® Bacterial Protein Extraction Reagent (ionic) is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves and safety goggles when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Technical Assistance

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.



Storage temperature



Do not use if package is damaged



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Disclaimer :

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