

PrepEase™ His-Tagged Protein Purification Kits – High Yield

Mini Kits - High Yield

Product Number 78800/1

Midi Kits - High Yield

Product Number 78802/3

Maxi Kits - High Yield

Product Number 78804/5

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STORAGE

Store at room temperature (20–25°C).

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.



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COMPONENTS

Always keep buffer bottles tightly closed.

PrepEase™ His-Tagged Protein Purification Mini Kit – High Yield

	10 preps 78800	50 preps 78801
PrepEase™ Ni-IDA Mini Columns	10	50
8X LEW Buffer	5 ml	25 ml
4X Elution Buffer	5 ml	20 ml

PrepEase™ His-Tagged Protein Purification Midi Kit – High Yield

	5 preps 78802	50 preps 78803
PrepEase™ Ni-IDA Midi Columns	5	50
8X LEW Buffer	30 ml	2 x 70 ml
4X Elution Buffer	25 ml	115 ml
Plastic Washers	5	50

PrepEase™ His-Tagged Protein Purification Maxi Kit – High Yield

	5 preps 78804	25 preps 78805
PrepEase™ Ni-IDA Maxi Columns	5	25
8X LEW Buffer	30 ml	2 x 70 ml
4X Elution Buffer	25 ml	115 ml
Plastic Washers	5	25

8X LEW Buffer (Lysis-Equilibrium-Wash Buffer): 400mM NaH₂PO₄, 2.4M NaCl, pH 8.0

4X Elution Buffer: 200mM NaH₂PO₄, 1.2M NaCl, 1.0M Imidazole, pH 8.0

STORAGE AND PREPARATION

All kit components may be stored at room temperature (20-25°C).

For purification under *Native Conditions*, simply dilute the supplied buffers in water to a 1X concentration.

1X LEW Buffer: 50mM NaH₂PO₄, 300mM NaCl, pH 8.0:

	Mini	Midi	Maxi
8X LEW Buffer	0.3 ml	1.5 ml	2.5 ml
Water	2.1 ml	10.5 ml	17.5 ml
Total 1X LEW Buffer needed per column	2.4 ml	12 ml	20 ml

1X Elution Buffer: 50mM NaH₂PO₄, 300mM NaCl, 250mM Imidazole, pH 8.0:

	Mini	Midi	Maxi
4X Elution Buffer	0.25 ml	1.5 ml	3.0 ml
Water	0.75 ml	4.5 ml	9.0 ml
Total 1X Elution Buffer needed per column	1 ml	6 ml	12 ml

For purification under *Denaturing Conditions*, dilute the supplied buffers to a 1X concentration and add Urea to a final concentration of 8M. Important: Prepare and use these buffers just before use due to the precipitation of urea over time.

1X LEW Denaturing Buffer: 50mM NaH₂PO₄, 300mM NaCl, pH 8.0, 8.0M Urea:

	10 ml	50 ml
8X LEW Buffer	1.25 ml	6.25 ml
Urea	4.8 gm	24 gm
Water	to 10 ml	to 50 ml

1X Elution Denaturing Buffer: 50mM NaH₂PO₄, 300mM NaCl, 250mM Imidazole, pH 8.0, 8.0M Urea:

	10 ml	50 ml
4X Elution Buffer	2.5 ml	12.5 ml
Urea	4.8 gm	24 gm
Water	to 10 ml	to 50 ml

MATERIALS NOT SUPPLIED

Lysozyme [USB PN 18645]

DNase I [USB PN 14365, or for rDNase I, USB PN 78311]

Deionized water

Urea (if using denaturing conditions) [USB PN 75826]

QUALITY CONTROL

All PrepEase™ Ni-IDA Columns are resistant to organic solvents such as alcohol, chloroform, and phenol and are free of contaminating proteases.

Physical Specifications:

Physical Form	Dry matrix, precharged with Ni ²⁺
Matrix	Macroporous silica
Density	0.5 gm/ml
Chelating Group	IDA (iminodiacetic acid)
Mean Particle Size	90 μm

SAFETY WARNINGS AND PRECAUTIONS

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Caution: All chemicals should be considered as potentially hazardous. We, therefore, recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing, such as lab coat, safety glasses, and gloves. Care should be taken to avoid contact with skin and eyes. In the case of contact with skin or eyes, wash immediately with water. See MSDS (Material Safety Data Sheet) for specific advice.

INTRODUCTION

Overview

The PrepEase™ Protein Purification Kits for High Specificity are designed to purify recombinant polyhistidine-tagged (His-tagged) recombinant proteins expressed in *E. coli* using immobilized metal ion affinity chromatography (IMAC). The kits may also be used to purify His-tagged proteins from other expression systems such as insect cells, mammalian, cells and yeast. Purification may take place under standard, native conditions or under denaturing conditions depending on the solubility of the expressed protein.

PrepEase™ Protein Purification Kits are based on the use of novel, dry silica-based resin, precharged with Ni²⁺ ions. Binding of protein is based on the interaction between the polyhistidine tag of the recombinant protein and immobilized Ni²⁺ ions. The High Yield Kits utilize IDA (iminodiacetic acid), a special chelating group, which enables strong and efficient binding of target proteins to the resin. IDA is a tri-dentate chelator which occupies three of the six binding sites in the coordination sphere of the Ni²⁺ ion. The remaining three coordination sites can be exchanged with histidine residues of the recombinant protein (Figure 1).

In contrast to traditional IDA matrices, PrepEase™ Ni-IDA Resin contains an optimized, low density of IDA ligands that are created by a special manufacturing process. The non-saturating surface concentration of IDA eliminates non-specific interactions with contaminating proteins. As a result, PrepEase™ Ni-IDA Resin allows for higher target protein purity.

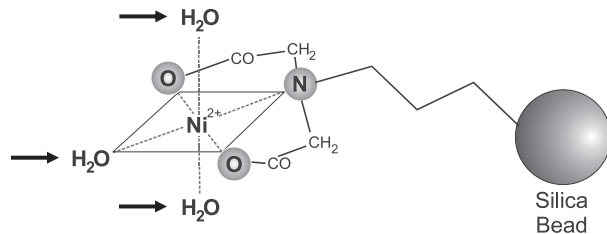


Figure 1. PrepEase™ Ni-IDA Resin – Structure of IDA complexed with Ni²⁺.

Purification Under Native Versus Denaturing Conditions

Ideally, target proteins remain soluble in the cytoplasm of *E. coli*. However, insoluble aggregates called inclusion bodies can form when some proteins are highly expressed. During purification of His-tagged proteins, bacterial cells are disrupted using lysozyme in combination with sonication followed by centrifugation. While the crude cell extracts will contain both soluble and insoluble target protein, only the soluble target protein is found in the supernatant. The clarified supernatant may be purified directly using the supplied PrepEase™ Ni-IDA Columns under native conditions using the standard protocol. In cases where the recombinant protein is insoluble with the formation of inclusion bodies, the target protein should first be extracted from the pellet under denaturing conditions using 8M urea. If the distribution of the recombinant protein is unknown, it is recommended to perform SDS-PAGE analysis using the crude cell extract and the clarified lysate before proceeding with the purification.

Binding Capacities of PrepEase™ Ni-IDA Columns

PrepEase™ Ni-IDA Column	Binding Capacity
Mini	800 µg
Midi	5 mg
Maxi	10 mg

The binding of His-tagged protein to PrepEase™ Ni-IDA Columns strongly depends on the characteristics of the protein itself, such as amino acid composition, molecular weight, 3-D structure, and oligomerization properties. Furthermore, the absolute yield also depends on the total amount and concentration of the target protein in the sample which directly correlates with the expression level and cell density of the expression culture. Therefore, binding capacity will vary for each His-tagged protein and may need to be empirically determined for each expression experiment.

For General Guidance on *E. coli* Culture and Buffer Volumes, please refer to the Supplementary Information section on p. 13.

Elution Procedures

Because virtually no contaminating host proteins bind to PrepEase™ Ni-IDA Columns, stringent washing procedures are not necessary. Therefore, the LEW Buffer which is used for cell lysis, column equilibration and washing, does not contain any imidazole. Bound His-tagged protein is competitively eluted off the column by adding imidazole, which is present in the supplied Elution Buffer. The PrepEase Elution Buffer contains 250mM imidazole to recover even, strong binding, multimeric proteins with more than one polyhistidine tag. Depending on the protein, elution may be effective in the presence of much lower imidazole concentrations (Figure 2) which will occur at the beginning of the gradient elution step.

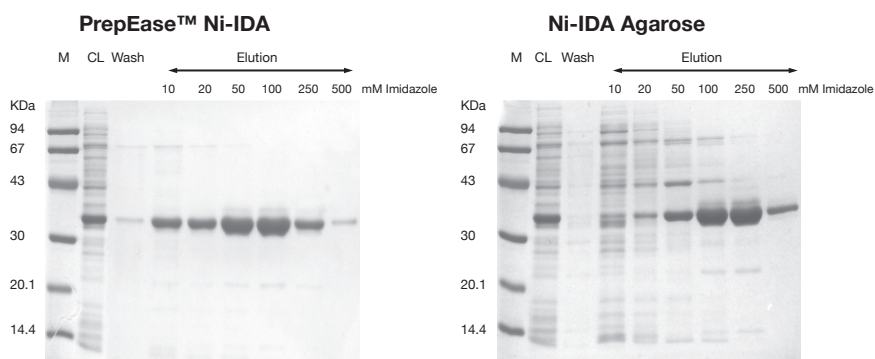


Figure 2. Purification of His-Tagged GFP using PrepEase™ Ni-IDA Resin.

Recombinant GFP was expressed in *E. coli*, lysed, loaded onto a PrepEase™ Ni-IDA Column and eluted with a stepwise imidazole gradient. Eluted fractions were analyzed by SDS-PAGE. Pure His-tagged protein may be eluted at much lower imidazole concentrations than from Ni-IDA agarose based columns (right panel). Ni-IDA agarose may require much higher concentrations of imidazole to effectively elute the protein. In addition, non-specific products are eluted with Ni-IDA agarose with imidazole concentrations ranging from 10mM to 100mM.

M = Marker, CL = Cleared Lysate.

PROTOCOLS

Purification of His-Tagged Proteins From *E. coli* Under Native Conditions

Note: This protocol is designed for purification of recombinant protein from high expressing cultures. For low expressing proteins refer to the General Guidance on *E. coli* Culture and Buffer Volumes in the Supplementary Information Section.

1. Recommended *E. coli* culture volumes

Prepare the following volumes of induced *E. coli* culture. Volume depends on expression system and characteristics of the His-tagged protein.

Mini 8-30 ml	Midi 50-200 ml	Maxi 100-375 ml
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2. Harvest bacterial cells

a. Harvest bacteria by centrifugation at **4,500 – 6,000 x g** for **15 min at 4°C**. Carefully discard supernatant.

b. Prepare 1X LEW Buffer and 1X Elution Buffer (see Storage and Preparation on p. 4 for additional information).

Cell pellets may be stored at -80°C until needed.

3. Resuspend bacterial pellet

Resuspend pellet in **1X LEW Buffer**. Perform this step on ice. Use 5 ml of 1X LEW Buffer for every 1 gm of wet cell pellet weight. Pipet up and down, or stir, until the pellet is completely resuspended with no visible cell aggregates.

If the cell pellet was frozen, thaw on ice with 1X LEW Buffer.

Mini 0.2-0.6 ml	Midi 1.0-3.5 ml	Maxi 2.0-7.5 ml
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4. Clarify lysate

a. Add **lysozyme** to a final concentration of **1 mg/ml**. Leave the solution on ice for **30 min** with occasional mixing if possible.

b. Sonicate the suspension on ice according to the instructions provided by the manufacturer. For example, use 10 x 15 second bursts with a 15 second cooling period between each burst.

c. If the lysate is still viscous due to incomplete fragmentation of DNA, add **5 µg/ml of DNase I**, pipette up and down or stir. Leave on ice for 15 min.

d. Centrifuge the crude lysate at **10,000 x g** for **30 min at 4°C** to remove cellular debris. Carefully transfer the supernatant to a clean tube without disturbing the pellet.

e. Store supernatant on ice.

If the supernatant is not clear, centrifuge a second time or filter through a 0.45 µm membrane (e.g. cellulose acetate) to avoid clogging the column with insoluble material.

5. Equilibrate PrepEase™ Ni-IDA Column

- Place column(s) into appropriate centrifuge tubes. (15 ml conical tube(s) work well.)
- Equilibrate columns with **1X LEW Buffer**. Allow columns to drain by gravity flow.

Mini 320 µl	Midi 2.0 ml	Maxi 4.0 ml
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6. Bind sample to PrepEase™ Ni-IDA Column

Add the clarified lysate to the column and allow column to drain by gravity flow.

7. Wash column

Wash column **2 times** with **1X LEW Buffer**. Allow column to drain by gravity flow. Discard eluate.

Mini 2 x 320 µl	Midi 2 x 2.0 ml	Maxi 2 x 4.0 ml
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8. Elute protein

Elute His-tagged protein into **3 separate collecting tubes** by adding **1X Elution Buffer**. Allow column to drain by gravity flow.

Mini 3 x 240 µl	Midi 3 x 1.5 ml	Maxi 3 x 3.0 ml
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9. Analyze fractions

To determine which fraction(s) contain the target His-tagged protein, analyze an aliquot of each by SDS-PAGE.

Dialyze as necessary to remove imidazole and establish proper storage conditions.

Purification of His-Tagged Proteins From *E. coli* Under Denaturing Conditions

Note: This modification to the standard protocol is performed when the recombinant His-tagged protein is insoluble due to the formation of inclusion bodies.

1. Follow the standard protocol up to Step 4d.

See above. This includes steps from 'Recommended *E. coli* culture volumes' to 'Clarify lysate'. Keep the cell pellet on ice.

2. Wash inclusion bodies

a. After the centrifugation step in 'Clarifying lysate', remove the supernatant and resuspend the cell pellet in **1X LEW Buffer** (see Storage and Preparation on p. 4 for additional information).

Mini 1.5 ml	Midi 7.5 ml	Maxi 15.0 ml
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b. Centrifuge the suspension at **10,000 x g** for **30 min** at **4°C**.

c. Discard supernatant.

3. Equilibrate PrepEase™ Ni-IDA Column

a. Prepare **1X LEW Denaturing Buffer** and **1X Elution Denaturing Buffer** (see Storage and Preparation on p. 4 for additional information).

b. Place column(s) into appropriate centrifuge tubes. (15 ml conical tube(s) work well.)

c. Equilibrate columns with **1X LEW Denaturing Buffer**. Allow columns to drain by gravity flow.

Mini 320 µl	Midi 2.0 ml	Maxi 4.0 ml
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4. Solubilize inclusion bodies

a. Resuspend cell pellet in **1X LEW Denaturing Buffer** to solubilize the inclusion bodies. Homogenization or sonication may be necessary to resuspend the pellet.

Mini 0.08-0.24 ml	Midi 0.4-1.5 ml	Maxi 0.8-3.0 ml
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b. Pipet up and down, or stir suspension on ice for **60 min** to dissolve the inclusion bodies.

c. Centrifuge the suspension at **10,000 x g** for **30 min** at **room temperature** to remove any remaining insoluble material.

d. Transfer supernatant to a clean tube without disturbing the pellet.

5. Bind sample to PrepEase™ Ni-IDA Column

Add supernatant to the equilibrated column and allow column to drain by gravity flow.

6. Wash column

Wash column **2 times** with **1X LEW Denaturing Buffer**. Allow column to drain by gravity flow.

Mini 2 x 320 µl	Midi 2 x 2.0 ml	Maxi 2 x 4.0 ml
---------------------------	---------------------------	---------------------------

7. Elute protein

Elute His-tagged protein into **3 separate collecting tubes** by adding **1X Elution Denaturing Buffer**. Allow column to drain by gravity flow.

Mini 3 x 240 µl	Midi 3 x 1.5 ml	Maxi 3 x 3.0 ml
---------------------------	---------------------------	---------------------------

8. Analyze fractions

To determine which fraction(s) contain the target His-tagged protein, analyze an aliquot of each by SDS-PAGE.

Dialyze as necessary to remove imidazole, urea, and establish proper storage conditions.

SUPPLEMENTARY INFORMATION

General Guidance on *E. coli* Culture and Buffer Volumes

Table 1. Determination of Cell Culture and Buffer Volumes for use with PrepEase™ Ni-IDA Columns

PrepEase™ Ni-IDA Column	Protein Expression ¹	His-tagged Protein Load (mg)	<i>E. coli</i> Culture Volume (ml)	<i>E. coli</i> Wet Pellet Mass (gm)	LEW Buffer for Cell Suspension (ml)	LEW Buffer Wash for Inclusion Bodies ² (ml)	LEW Denaturing Buffer ³ (8M Urea) (ml)
Mini	High 100 mg/ml	0.8 - 3.0	8.0 - 30	0.04 - 0.12	0.2 - 0.6	0.4 - 1.2	0.08 - 0.24
	Low 10 mg/ml	0.8 - 3.0	80 - 300	0.4 - 1.2	0.8 - 2.4	4.0 - 12	0.8 - 2.4
Midi	High 100 mg/ml	5 - 20	50 - 200	0.2 - 0.75	1.0 - 3.75	2.0 - 7.5	0.4 - 1.5
	Low 10 mg/ml	5 - 20	500 - 2000	2.0 - 7.5	4.0 - 15	20 - 75	4.0 - 15
Maxi	High 100 mg/ml	10 - 38	100 - 375	0.4 - 1.5	2.0 - 7.5	4.0 - 15	0.8 - 3.0
	Low 10 mg/ml	10 - 38	1000 - 3750	4.0 - 15	8.0 - 30	40 - 150	8.0 - 30

1 – High protein expression: 5 ml 1X LEW Buffer per 1 gm of wet pellet

Low protein expression: 2 ml 1X LEW Buffer per 1 gm of wet pellet

2 – 10 ml 1X LEW Buffer per 1 gm of wet pellet for washing inclusion bodies in denaturing protocol

3 – 2 ml 1X LEW Denaturing Buffer per 1 gm wet pellet in denaturing protocol

Reagent Compatibility Chart

Reagent	Effect	Comments
Sodium Phosphate	Used in LEW and Elution Buffer in order to buffer the solutions at pH 8	50mM is recommended. The pH of any buffer should be adjusted to 8, although in some cases pH 7 and 8 can be used
Tris	Coordinates with Ni ²⁺ ions, causing a decrease in capacity	10mM may be used, sodium phosphate buffer is recommended
Sodium Chloride	Prevents ionic interactions and non-specific binding	Up to 2M but at least 0.3M should be used
Imidazole	Binds to immobilized Ni ²⁺ ions and competes with poly-his-tagged proteins	Do not include in LEW Buffer
Urea	Solubilizes protein	Use 8M for purification under denaturing conditions
GuHCl	Solubilizes protein	Up to 6M can be used
β-Mercaptoethanol	Prevents formation of disulfide bonds, can reduce Ni ²⁺ ions at higher concentrations	Up to 50mM in samples has been used successfully
DTT, DTE	Can reduce Ni ²⁺ ions at higher concentrations	Up to 10mM in samples has been used successfully
Glutathione Reduced	Can reduce Ni ²⁺ ions at higher concentrations	Up to 30mM in samples has been used successfully
Glycerol	Prevents hydrophobic interactions between proteins	Up to 50% can be used
EDTA	Coordinates with Ni ²⁺ ions, causing a decrease in capacity at higher concentrations	Not recommended, but up to 1mM has been used successfully in some cases
Ethanol	Prevents hydrophobic interactions between protein	Up to 20% can be used; Ethanol can precipitate proteins, causing low flow rates and clogging of columns
SDS	Interacts with Ni ²⁺ ions, causing a decrease in capacity	Not recommended, but up to 0.05% in samples has been used successfully
Triton®, Tween®	Removes background proteins	Up to 2% can be used

TROUBLESHOOTING

Problem Possible Causes and Solutions

Sample does not enter column bed

1. Sample/lysate contains insoluble material

If lysate is not clear, perform centrifugation or filtration to avoid clogging of the PrepEase™ Ni-IDA Column.

2. Sample/lysate contains genomic DNA

Lysate may remain viscous from incomplete shearing of genomic DNA after sonication. Add 5 µg/ml DNase I and incubate on ice for 10 min.

Protein does not bind to the resin

1. Problem with vector construction

Ensure that protein and his-tag are in frame.

2. Incorrect binding conditions

Check composition of buffers and verify pH 7-8. Ensure absence of chelating or strong reducing agents or imidazole in LEW Buffer.

Protein elutes with wash buffer

1. Incorrect binding conditions

Check composition of buffers and verify pH 7-8. Ensure absence of chelating or strong reducing agents or imidazole in LEW Buffer.

Protein does not elute from column

1. Elution conditions are too mild

Increase concentration of imidazole.

Elution of His-tagged protein contains non-specific products

1. Insufficient wash

Use larger volumes for washing step.

2. Binding and wash conditions are too mild

Add small amounts of imidazole (1-10mM). Verify that the imidazole concentration is low enough to allow binding of the His-tagged protein.

3. Contaminating proteins and target proteins are linked together via disulfide bonds

Add up to 30mM β -mercaptoethanol to reduce disulfide bonds.

4. Contaminating proteins are proteolytic products of target protein

Perform cell lysis at 4°C.

Include protease inhibitors.

5. Expression is too low

Very low amounts of His-tagged protein are not able to compete with or replace the majority of contaminating proteins effectively.

Increase expression level.

Increase amount of starting cell material.

Do not exceed recommended lysis volumes.

If problems persist please contact USB Technical Support for assistance at (800) 321-9322 or techsupport@usbweb.com. For technical support outside the U.S., please visit our website for up-to-date contact information on the USB product distributor within your area.

REFERENCES

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3. PORATH, J., CARLSSON, J., OLSSON, I., AND BELFRAGE, G. (1975) *Nature* **258**, 598-599.
4. SAMBROOK, J., FRITSCH, E. F., AND MANIATIS, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
5. SMITH, M. C., FURMAN T. C., INGOLIA T. D., AND PIDGEON C. (1988) *J. Biol. Chem.* **263(15)** 7211-7215.

RELATED PRODUCTS

Protein Purification Products

Product	Application	Pack size	Product number
PrepEase™ His-Tagged Protein Purification Mini Kits - High Specificity	Protein purification	10 preps 50 preps	78790 78791
PrepEase™ His-Tagged Protein Purification Midi Kits - High Specificity	Protein purification	5 preps 50 preps	78792 78793
PrepEase™ His-Tagged Protein Purification Maxi Kits - High Specificity	Protein purification	5 preps 25 preps	78794 78795
PrepEase™ His-Tagged Protein Purification Resin - High Specificity	Protein purification	5 gm 30 gm 120 gm	78796
PrepEase™ His-Tagged Protein Purification Resin - High Yield	Protein purification	5 gm 30 gm 120 gm	78806
PrepEase™ His-Tagged Protein Purification Sample Kit - High Specificity & High Yield	Protein purification	1 column each	78810

Ultrapure Reagents

Product	Application	Pack size	Product number
Acrylamide	Gel electrophoresis	25 gm 100 gm 500 gm 1 kg	75820
Ammonium Persulfate (APS)	Gel electrophoresis	10 gm 100 gm 1 kg	76322
Dithiothreitol (DTT)	Reducing agent	1 gm 5 gm 25 gm 100 gm	15397
Dithiothreitol (DTT), 0.1M Solution	Reducing agent	150 μ l	70726
Imidazole	Protein elution	25 gm 100 gm 500 gm 1 kg 5 kg	17525
LB Broth, Ready-Made Powder	Bacterial culture	250 gm 1 kg	75852
N,N'-Methylene-bis-Acrylamide	Gel electrophoresis	25 gm 50 gm 100 gm 1 kg	75821

Product	Application	Pack size	Product number
RapidGel™, 40% Liquid Acrylamide Stock Solution	Gel electrophoresis	500 ml	75848
Sodium Dodecyl Sulfate (SDS), 10% Solution	SDS PAGE Protein solubilization	100 ml 500 ml 1 L	77504
TAE Buffer, 10X Solution	Gel electrophoresis	1 L 5 L	75904
TBE Buffer, 5X Solution	Gel electrophoresis	1 L 5 L	75891
TBE Buffer, 10X Ready-Mixed Powder	Gel electrophoresis	6 x 200 ml	70454
TE Buffer, 1X Solution	Storage of DNA	10 x 1 ml 100 ml 500 ml	75893
Urea	Denaturant	1 kg 5 kg	75826
Water, Nuclease-Free	Resuspending plasmid Buffer preparation DNA storage	10 x 1 ml 100 ml 500 ml 1 L 5 L	71786

Enzymes

Product	Application	Pack size	Product number
Lysozyme	Bacterial cell lysis	1 gm 5 gm 10 gm 25 gm 100 gm	18645
DNase I, Lyophilized	DNA digestion	5 mg	14365
rDNase I, RNase-free	DNA digestion	1000 units 2500 units	78311

Markers

Product	Application	Pack size	Product number
Protein Markers, 10 – 225 kDa	Size determination	500 µl	76740
Western Markers Mid-Range I	Size determination	5 x 40 µl	30172

Ultrapure Antibiotics

Product	Application	Pack size	Product number
Ampicillin, Sodium Salt	Cell culture	5 gm 25 gm 100 gm	11259
Chloramphenicol	Cell culture	5 gm 25 gm 100 gm 1 kg	23660
Kanamycin Sulfate	Cell culture	5 gm 25 gm	17924
Streptomycin Sulfate	Cell culture	10 gm 25 gm 100 gm 1 kg	21865
Tetracycline Hydrochloride	Cell culture	25 gm 100 gm 1 kg	22105

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Material Safety Data Sheet

Revision: 02/13/2006

Hazard information is provided for compliance with both the UK Chemicals (Hazard Information and Packaging) (CHIP) Regulations and the US Hazard Communication Standard (HCS)

IDENTIFICATION OF THE PRODUCT NAME:

SUBSTANCE/PREPARATION PrepEase™ His-Tagged Protein Purification Mini Kit - High Yield
AND COMPANY PrepEase™ His-Tagged Protein Purification Midi Kit - High Yield
 PrepEase™ His-Tagged Protein Purification Maxi Kit - High Yield

SUPPLIER:

USB Corporation
 26111 Miles Road, Cleveland, Ohio 44128 Phone: (216) 765-5000

Please visit our website at www.usbweb.com for contact information on USB product distributors within your area.

PRODUCT CODE: EEC NUMBER:

78800 / 78801 206-019-2
 78802 / 78803 (Imidazole)
 78804 / 78805

EMERGENCY CONTACT:

Chemtrec: (800) 424-9300
Outside USA & Canada: 703-527-3887

COMPOSITION/

HAZARDOUS COMPONENTS

HAZARD	CAS NO.	%WT	TLV	CHIP R & S Phrases
Imidazole (Elution Buffer)	288-32-4	~7%	—	R:36/37/38 Irritating to eyes, respiratory system and skin. S:26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S:36/37/39 Wear suitable protective clothing, gloves and eye/face protection. S:45 In case of accident or if you feel unwell seek medical advice immediately (show the label where possible).

HAZARDS IDENTIFICATION

CHIP
 Irritant
HCS
 Irritant

FIRST-AID MEASURES

EYES: Flush with water for 15 minutes. Seek medical advice if irritation persists.
SKIN: Flush with water, then wash thoroughly with soap and water. Remove contaminated clothing and wash before reuse. Seek medical attention if irritation persists.
INHALATION: Remove the victim from exposure and move to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Keep victim quiet and warm. Seek immediate medical attention.
INGESTION: Drink water and seek immediate medical attention. Avoid alcoholic beverages. Never give anything by mouth to an unconscious person.

FIRE-FIGHTING INFORMATION

Use media suitable to extinguish the supporting or surrounding fire. Wear NIOSH (or equivalent) approved self contained breathing apparatus. For small fires only: use carbon dioxide, dry powder or foam. Emits toxic fumes under fire conditions. NFPA Rating: H=1 F=0 R=0.
 Flash Point (Pure Imidazole) = 145°C (Closed Cup). Autoignition Temperature (Pure Imidazole) = 480°C.

ACCIDENTAL RELEASE MEASURES

Wear appropriate personal protective equipment and clothing including lab coat, safety goggles, gloves and NIOSH-approved respirator. Collect in a manner that does not create dust and place in a suitable waste container. Avoid contact of material with skin or eyes. Use adequate ventilation.

HANDLING AND STORAGE

Wear appropriate personal protective equipment and clothing including lab coat, safety goggles, gloves and NIOSH-approved respirator. Avoid contact of material with skin or eyes. Store ambient away from incompatible materials.

PERSONAL PROTECTION

Wear appropriate personal protective equipment and clothing including lab coat, safety goggles, gloves and NIOSH-approved respirator. A qualified industrial hygienist should evaluate the need for respiratory protection. Use respiratory protection approved by NIOSH (or equivalent) and appropriate to the hazard. Avoid contact of material with skin or eyes. Mechanical ventilation or local exhaust as needed to control exposure to dust, vapors or mists. Access to a safety shower and eye-wash. HMIS Rating: H=1 F=0 R=0.

PHYSICAL AND CHEMICAL PROPERTIES

Appearance: Kit containing columns and buffers	Boiling Point: No data available
Vapor Pressure: No data available	Vapor Density: No data available
Solubility (Water): Soluble	Specific Gravity: No data available
Percent Volatile: No data available	Evaporation Rate: No data available
Chemical Formula: Kit	

STABILITY AND REACTIVITY

Product is stable. Hazardous decomposition products may include carbon monoxide, carbon dioxide, hydrogen cyanide, nitrogen oxides and ammonia. Incompatible with strong oxidizing agents, alkalis, acids and acid anhydrides. Hazardous polymerization will not occur.



TOXICOLOGICAL INFORMATION**EFFECTS OF OVEREXPOSURE:**

EYES: Contact causes irritation.

SKIN: Contact causes irritation.

INHALATION: Material is irritating to mucous membranes and upper respiratory tract.
INGESTION: Ingestion may cause severe irritation to the mouth, throat, esophagus and gastrointestinal tract.

ADDITIONAL INFORMATION:

May be harmful by inhalation, ingestion or skin absorption. May cause irritation and/or burns to the eyes, respiratory system and skin. For Imidazole: Laboratory experiments have resulted in mutagenic effects. May cause adverse reproductive effects. Animal evidence suggests that fetotoxicity and teratogenicity may be observed at doses that also cause harm in the mothers. Reproductive effects, mutation and toxicity data for Imidazole listed in RTECS under NI3325000.

Only select RTECS information is provided here. Please see actual RTECS entry for complete information.

Oral Rat LD50 = 220 mg/kg (Warsaw, Poland - 1993). Details of toxic effects not reported other than lethal dose value.

Reproductive: Paternal effects - impotence (1990).

Definitions: RTECS = Registry of Toxic Effects of Chemical Substances.

No information available.

ECOLOGICAL INFORMATION**DISPOSAL CONSIDERATIONS**

Dispose of material in accordance with applicable local, state, and federal regulations.

TRANSPORTATION INFORMATION

US DOT / IATA: No applicable information.

REGULATORY INFORMATION

RCRA - No applicable information.

SARA 302 - This material does not have an RQ or TPQ.

SARA 313 - This material is not reportable under 313.

EPA TSCA Section 8(b) - Imidazole: Chemical Inventory.

8(d) - Imidazole: unpublished health/safety studies.

Exposure Limits - Not established.

California Proposition 65 - No applicable information.

This data sheet is based upon information believed to be reliable. The Company makes no statement or warranty as to the accuracy or completeness of the information contained herein which is offered for your consideration, investigation and verification. Any use of the information contained in this data sheet must be determined by the user to be in accordance with appropriate applicable regulations.