

PrepEase™ His-Tagged Protein Purification Kits – High Specificity

Brief Protocol

This protocol is designed for purification of recombinant protein under **native conditions from high expression protein cultures**. For purification from low expression protein cultures, refer to the Protocol Booklet supplied with the kit.

STEP	Mini PN 78790/91	Midi PN 78792/93	Maxi PN 78794/95
------	---------------------	---------------------	---------------------

1. Cultivate *E. coli* culture

Prepare induced *E. coli* in LB culture medium.

Mini	Midi	Maxi
5 - 25 ml	25 - 50 ml	50 - 300 ml

2. Harvest bacterial cells



- Centrifuge the lysate at 4,500 – 6,000 x g for 15 min at 4°C. Carefully discard supernatant.
- Prepare 1X LEW Buffer and 1X Elution Buffer.
Cell pellets may be stored at -80°C until needed.

3. Resuspend bacterial pellet



Resuspend pellet in LEW Buffer. Perform on ice. Use 5 ml of 1X LEW Buffer for every 1 gm of wet cell pellet weight.

Mini	Midi	Maxi
0.1 - 0.5 ml	0.5 - 3.0 ml	1.0 - 6.0 ml

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

4. Clarify lysate



- Add lysozyme to a final concentration of 1 mg/ml.
- Leave on ice for 30 min with occasional mixing if possible.
- Sonicate suspension on ice. For example, use 10 x 15 second bursts with a 15 second cooling period between each burst.

Continued on back side.



- If lysate is still viscous, add 5 µg/ml DNase I. Pipette up and down or stir. Keep on ice for 15 min.

- Centrifuge the lysate at 10,000 x g for 30 min at 4°C.

- Transfer supernatant to a clean tube.

5. Equilibrate PrepEase™ Ni-TED Column



- Place column into a 15 ml conical centrifuge tube.
- Equilibrate column with 1X LEW Buffer.
- Allow column to drain by gravity flow.

Mini	Midi	Maxi
320 µl	2.0 ml	4.0 ml

6. Bind sample to PrepEase™ Ni-TED Column



Add cleared lysate to column and allow column to drain by gravity flow.

7. Wash column



Wash column 2 times with 1X LEW Buffer and allow column to drain by gravity flow.

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

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	Midi	Maxi
3 x 240 µl	3 x 1.5 ml	3 x 3 ml

9. Analyze fractions

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

For the protocol on **Purification Under Denaturing Conditions** see the protocol booklet.

USB and the logo design are registered trademarks of USB Corporation. PrepEase is a trademark of USB Corporation. ©USB Corporation 2006—All rights reserved.
USB Corporation
 26111 Miles Road, Cleveland, Ohio 44128 USA
www.usbweb.com

P-78790B
 rev 03/06