# Antagene, Inc. Product Information

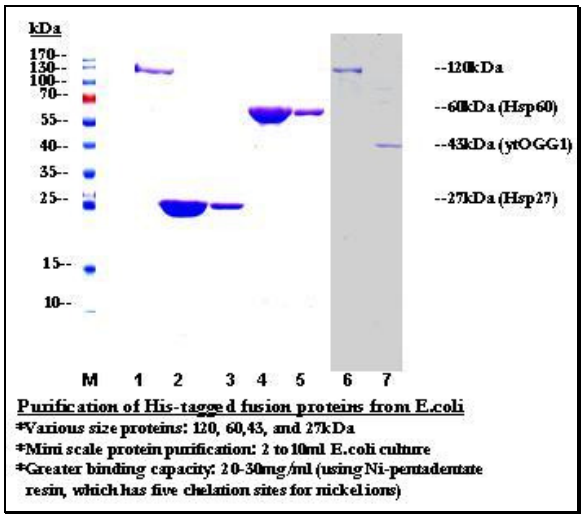
**Product Name: EZ E.coli His-tag protein miniprep kit**
**Catalog no.: ANT-EZPEHis101**
**Contents: Ni-pentadentate resin, His binding, washing, elution buffer, E.coli lysis buffer**
**Size: 10 and 50 minipreps**

**Feature:**
-Easy and fast method for His-tagged protein purification in 30 minutes.
-Mini-scale protein expression and purification protocol development for multiple samples.
-High quality purified proteins for enzyme activity assay and other applications.
-High protein binding capacity by using Ni-pentadentate resin (20-30mg/ml): load 3mg protein per 150ul resin.
-Low elution volume (100-200ul) for higher protein concentration.

**Storage: 4*C**

**Components for the reagents:**

| | EZPHis101-10 | EZPHis101-50 |
|---|---|---|
| Spin columns | 10 | 50 |
| Collection tubes | 10 | 50 |
| Ni resin (50%), ml | 2.0 | 10 |
| His binding buffer, ml | 10 | 50 |
| His wash buffer, ml | 10 | 50 |
| His elution buffer, ml | 5 | 25 |
| E.coli Lysis buffer, ml | 3.5 | 16 |



Purification of His-tagged fusion proteins from E.coli
*Various size proteins: 120, 60,43, and 27kDa
*Mini scale protein purification: 2 to 10ml E.coli culture
*Greater binding capacity: 20-30mg/ml (using Ni-pentadentate resin, which has five chelation sites for nickel ions)

**Instructions for use:**

1. Harvest 4 ml E.coli culture with polyhistidine-tagged gene and resuspend in 0.5 -1 ml His binding buffer. Cell can be lysed by sonication, French press, or repeated freeze-thaw cycles. You can also use other commercially available extraction buffer to lyse the cell (see the following Option in step 2). Spin the sample at 12,000g at 4*C for 10 min to remove the cell debris. Keep the supernatant on ice and save the pellet if your protein is insoluble.
2. (Option) Add 0.3 ml of E.coli lysis buffer to the pellet from 2 ml of E.coli culture. Re-suspend the pellet by Votex.
3. Transfer 150ul of Ni resin to the spin column with a collection tube. Note: shake the bottle to make sure the resin is fully re-supended and use 1ml pipette tip to transfer the resin into column.
4. Spin the resin for 5 seconds in a microcentrifuge (1,000rpm) to remove the resin storage buffer.
5. Load 200 to 400 ul of cell free extract (from step 1 or 2) to the column and re-suspend the gel by tapping the column or using vortex (make sure to mix the resin very well).
6. Leave the column at room temperature for 2-5 minutes to allow the His-tagged protein binding to the Ni resin.
7. Spin the column with collection tube for 5 seconds (1,000rpm). Discard or save the FT for gel check if necessary.
8. Add 250 ul of wash buffer and re-suspend the gel by tapping the column or using vortex (make sure to mix the resin very well). Centrifuge 5 seconds (1,000rpm) to remove the washing buffer. Repeat this step1or 2 times for lower background.
9. Place the column into a clean microcentrifuge tube and add 150 ul elution buffer to the column. Again re-suspend the gel by tapping the column or using vortex (make sure to mix the resin very well) and spin for 5 seconds at 1,000rpm. Save the flow though and keep at -80*C for long term storage.
10. Check the protein purification by SDS PAGE gel or western blot.
11. You can also measure protein concentration and check the protein activity by its functional assay.

**Solutions:**

His binding buffer: 50mM Tris/HCl (pH7.5), 300mM NaCl, 10% glycerol, 2.5mM bme
His wash buffer: 50mM Tris/HCl (pH7.5), 300mM NaCl, 10% glycerol, 2.5mM bme, 50mM Imidazole
His elution buffer: 50mM Tris/HCl (pH7.5), 300mM NaCl, 10% glycerol, 2.5mM bme, 250mM Imidazole
Ni-pentadentate resin (50%)

**FOR RESEARCH USE ONLY**

Antagene Inc
Toll Free: 1-866-964-2589; Fax: 650-964-2519; Web: www.antageneinc.com