QIAGEN Supplementary Protocol:

Purification of 6xHis-tagged proteins using the BioSprint® 96

This protocol is for purification of 6xHis-tagged proteins from up to 96 samples of crude or cleared *E. coli* lysate using the BioSprint 96.

Introduction

The BioSprint 96 uses Ni-NTA–coated magnetic agarose beads for rapid purification of recombinant proteins. Ni-NTA Magnetic Agarose Beads combine the proven efficiency of Ni-NTA–based protein purification with the convenient handling of magnetic beads. Proteins bind via their 6xHis tag to the Ni-NTA–coated surface of the magnetic beads. The magnetic beads are then efficiently washed, removing nonspecifically bound protein while leaving the tagged protein on the matrix. Pure protein of interest is finally eluted in a small volume delivering high-purity protein preparations at a concentration suitable for functional analyses. BioSprint 96 Protein procedures are designed for purification of 6xHis-tagged proteins in a single-step procedure. Purification of up to 96 samples under native or denaturing conditions can be carried out in less than 45 minutes. Crude or cleared lysates from *E. coli* or eukaryotic cells are used as starting material.

Note: BioSprint 96 instruments purchased before 1 December 2005 will need to have the protocol "BS96 Ni_NTA" installed. For more information, please contact one of the QIAGEN Technical Service Departments or local distributors.

IMPORTANT: Please read the *BioSprint 96 User Manual*, paying careful attention to the safety information, before beginning this procedure.

Storage

Ni-NTA Magnetic Agarose Beads are supplied as a 5% (v/v) suspension in 30% ethanol and should be stored at 2–8°C. They can be stored under these conditions for up to 6 months without any reduction in performance.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN® kit and kit component.

The following risk and safety phrases apply to Ni-NTA Magnetic Agarose Beads:

Ni-NTA Magnetic Agarose Beads

Contains nickel-nitrilotriacetic acid, ethanol: Harmful, sensitizer, flammable. Risk and safety phrases*: R10-22-40-42/43 S13-26-36-46.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- BioSprint 96, cat. no. 9000852
- Magnetic head for use with large 96-rod covers (supplied with the BioSprint 96)
- Large 96-Rod Cover (16), cat. no. 1031668
- 96-Well Microplates MP (20), cat. no. 1031656
- S-Blocks (24), cat. no. 19585
- Ni-NTA Magnetic Agarose Beads (2 x 1 ml or 6 x 1 ml), cat. no. 36111 or 36113, respectively
- Lysis, wash, and elution buffers and other reagents (see individual protocols and appendix for details)
- Pipettors and disposable pipet tips with aerosol barriers (20–1000 µl)
- Multichannel pipettor and disposable pipet tips with aerosol barriers (e.g., Finnpipette® Digital and Finntip® Filters from Thermo Electron, see www.thermo.com)†
- Soft cloth or tissue and 70% ethanol or other disinfectant to clean the worktable
- Disposable gloves

* R10: Flammable; R22: Harmful if swallowed; R40: Limited evidence of a carcinogenic effect; R42/43: May cause sensitization by inhalation and skin contact; S13: Keep away from food, drink, and animal feedingstuffs; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S46: If swallowed, seek medical advice immediately and show this container or label.

† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important notes

Starting material and elution volumes

BioSprint 96 Protein procedures are designed to purify proteins from individual small-scale cell cultures (for *E. coli*, up to 10 ml per well can be processed). The amount of starting material and elution volume ranges used in BioSprint protein applications are shown in Table 1. The indicated default values are programmed into the BioSprint software. If users wish to alter these values, they must be changed in the respective protocol, the protocol saved under a new file name, and exported to the BioSprint instrument. For each procedure, sample and elution volumes can be adjusted within the ranges shown to give a yield and concentration of high-purity protein suitable for subsequent applications.

Table 1. Sample, Wash, and Elution Volumes used in BioSprint 96 Protein Procedures

Culture volume	Lysate volume per well (µl)	Wash buffer volume (µl)	Elution step volume (µl)*
Up to 10 ml	500–1000	2 x 500	50† – 200

* Two elution steps are performed. † Default value per elution step when using 50 µl bead suspension.

Amount of bead suspension

Where possible, the amount of bead suspension used in BioSprint 96 protein purifications should be adjusted to the expected yield of purified protein. Table 2 shows typical binding capacities of different volumes of bead suspension. The values given are based on those obtained for a globular protein 25–50 kDa in size. Values may vary depending on protein conformation.

Table 2. Protein Binding Capacities of Ni-NTA Magnetic Beads

Matrix	Binding capacity	50 µl suspension	100 µl suspension	200 µl suspension
Ni-NTA Magnetic Agarose Beads	0.5–1 µg protein/µl suspension	25–50 µg 6xHis-tagged protein	50–100 µg 6xHis-tagged protein	100–200 µg 6xHis-tagged protein

Preparation of lysates

6xHis-tagged proteins can be purified under native or denaturing conditions using the BioSprint 96. If crude lysates are used, high viscosity caused by genomic DNA must be reduced by nuclease digestion.

Protocol: Generation of crude *E. coli* lysates containing 6xHis-tagged proteins

This protocol gives instruction for the preparation of crude lysates for purification under ■ native or ◆ denaturing conditions. Buffer compositions are provided in the appendix, pages 9–10.

Reagents to be supplied by user

- Lysis buffer ■ NPI-10-T-L or ◆ Buffer B-7M urea-T
- Benzonase® (purity grade I, 25 U/µl, Merck, Germany, cat. no. 1.01694.0001)

Procedure

1. **Thaw cell pellet for 15 min on ice and resuspend cells in 500 µl lysis buffer ■ NPI-10-T-L or ◆ Buffer B-7M urea-T.**
2. **Add 3 units of Benzonase per ml *E. coli* culture volume processed.**

   For example, to process a pellet originating from a 10 ml culture, add 3 x 10 = 30 units Benzonase.
3. **■ Native conditions: Incubate on ice for 30 min.**

   To ensure efficient cell lysis by lysozyme, cells should be incubated on ice for at least 30 min. Save an aliquot of the lysate for SDS-PAGE analysis if desired.

   ◆ Denaturing conditions: Incubate on an end-over-end shaker for 30 min at room temperature (15–25°C).

   Save an aliquot of the lysate for SDS-PAGE analysis if desired.
4. **Proceed to the purification protocol on page 6.**

Protocol: Generation of cleared *E. coli* lysates containing 6xHis-tagged proteins

This protocol gives instruction for the preparation of cleared lysates for purification under ■ native or ◆ denaturing conditions. Buffer compositions are provided in the appendix, pages 9–10.

Reagents to be supplied by user

■ Lysis buffer ■ NPI-10-T-L or ◆ Buffer B-T

Procedure

1. **Thaw cell pellet for 15 min on ice and resuspend cells in 500 µl lysis buffer ■ NPI-10-T-L or ◆ Buffer B-T.**

2. **■ Native conditions: Incubate on ice for 30 min.**

   To ensure efficient cell lysis by lysozyme, cells should be incubated on ice for at least 30 min. Save an aliquot of the lysate for SDS-PAGE analysis if desired.

   ◆ Denaturing conditions: Incubate on an end-over-end shaker for 30 min at room temperature (15–25°C).

   Save an aliquot of the lysate for SDS-PAGE analysis if desired.

3. **Centrifuge lysate at 10,000 x *g* for 20–30 min at 4°C to pellet the cellular debris. Save the supernatant (cleared lysate).**

   Save an aliquot of the lysate for SDS-PAGE analysis if desired.

4. **Proceed to the purification protocol on page 6.**

Protocol: Purification of 6xHis-tagged proteins using the BioSprint 96

Important points before starting

- Ensure that you are familiar with operating the BioSprint 96. Refer to the *BioSprint 96 User Manual* for operating instructions.
- All samples in a single run should have the same volume. If the volume of samples needs to be increased, add the appropriate volume of buffer NP-10-T.

Procedure

1. **Prepare three S-Blocks and three 96-well microplates according to the following table.**
   In each plate or block, the number of wells to be filled with buffer should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1–H1 to A6–H6 of each plate or block).

Slot	Plate/block	Native conditions	Denaturing conditions	Volume to add per well (µl)
6	96-well microplate MP	Large 96-rod cover	Large 96-rod cover	–
5	96-well microplate MP	Buffer NPI-250-T	Buffer E-T	75
4	96-well microplate MP	Buffer NPI-250-T	Buffer E-T	75
3	S-Block	Buffer NPI-20-T	Buffer C-T	500
2	S-Block	Buffer NPI-20-T	Buffer B-T	500
1	S-Block	Crude or cleared lysate	Crude or cleared lysate	500
		Ni-NTA Magnetic Agarose Beads*	Ni-NTA Magnetic Agarose Beads*	50

* Resuspend Ni-NTA Magnetic Agarose Beads by pipetting up and down or by vortexing for 2 s before pipetting into the S-Block.

2. **Switch on the BioSprint 96 at the power switch.**
3. **Slide open the front door of the protective cover.**

4. **Select the protocol "BS96 Ni_NTA" using the ^ and v keys on the BioSprint 96. Press "Start" to start the protocol run.**

5. **The LCD displays a message asking you to load slot 6 of the worktable with the 96-rod cover (see table below). After loading slot 6, press "Start". The worktable rotates and a new message appears, asking you to load slot 5 with the elution plate. Load slot 5 and press "Start" again. Continue this process of pressing "Start" and loading a particular slot until all slots are loaded.**

   Note: Each slot is labeled with a number. Load each 96-well plate or S-Block so that well A1 is aligned with the slot's label (i.e., well A1 faces inward).

Slot	Message when loading	Plate/block	Content	Volume per well (µl)
6	Load Rod Cover	96-well microplate MP	Large 96-rod cover	–
5	Load Elution 2	96-well microplate MP	Elution Buffer	75
4	Load Elution 1	96-well microplate MP	Elution Buffer	75
3	Load Wash 2	S-Block	Wash Buffer 2	500
2	Load Wash 1	S-Block	Wash Buffer 1	500
1	Load Sample	S-Block	Crude or cleared lysate	500
			Ni-NTA Magnetic Agarose Beads	50

6. **Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.**

   Warning: Avoid contact with moving parts during operation of the BioSprint 96. See the *BioSprint 96 User Manual* for safety information.

7. **Press "Start" to start sample processing.**

8. **After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press "Start" after removing each plate or block. The first item to be removed contains the purified samples.**

9. **Press "Stop" after all plates and blocks are removed.**

10. **Discard the used plates, blocks, and 96-rod cover according to your local safety regulations.**

    Note: See "Safety Information", page 1.

11. **Switch off the BioSprint 96 at the power switch.**

12. **Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.**

    Note: Do not use bleach as disinfectant. See "Safety Information", page 1.

Appendix

Buffers for purification of 6xHis-tagged proteins from *E. coli* cell lysates under native conditions using Ni-NTA Magnetic Agarose Beads

NPI-10-T-L (Ni-NTA Beads Lysis Buffer, 1 Litre):

50 mM NaH_2PO_4	6.90 g $NaH_2PO4{\cdot}H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
10 mM imidazole	0.68 g imidazole (MW 68.08 g/mol)
0.05% (v/v) Tween® 20	5 ml of a 10% (v/v) Tween 20 stock solution

Adjust pH to 8.0 using NaOH.

Complete the buffer by addition of 1 mg/ml lysozyme (50,000 units). Dissolve 10 mg lysozyme powder (e.g., Roche cat. no. 837059) in 10 ml buffer.

NPI-20-T (Ni-NTA Beads Wash Buffer, 1 Liter):

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4{\cdot}H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
20 mM imidazole	1.36 g imidazole (MW 68.08 g/mol)
0.05% (v/v) Tween 20	5 ml of a 10% (v/v) Tween 20 stock solution

Adjust pH to 8.0 using NaOH.

NPI-250-T (Ni-NTA Beads Elution Buffer, 1 Liter):

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4{\cdot}H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
250 mM imidazole	17.0 g imidazole (MW 68.08 g/mol)
0.05% (v/v) Tween 20	5 ml of a 10% (v/v) Tween 20 stock solution

Adjust pH to 8.0 using NaOH.

Buffers for purification of 6xHis-tagged proteins from *E. coli* cell lysates under denaturing conditions using Ni-NTA Magnetic Agarose Beads

Buffer B-7M urea-T (denaturing lysis buffer for crude lysates, 1 liter):

7 M Urea	420.42 g urea (60.06 g/mol)
100 mM NaH_2PO_4	13.80 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
10 mM Tris·Cl	12.10 g Tris·Cl (MW 121.1 g/mol)
0.05% (v/v) Tween 20	5 ml of a 10% (v/v) Tween 20 stock solution

Adjust pH to 8.0 using HCl.

Buffer B-T (denaturing lysis buffer for cleared lysates, 1 Liter):

8 M Urea	480.50 g urea (60.06 g/mol)
100 mM NaH_2PO_4	13.80 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
10 mM Tris·Cl	12.10 g Tris·Cl (MW 121.1 g/mol)
0.05% (v/v) Tween 20	5 ml of a 10% (v/v) Tween 20 stock solution

Adjust pH to 8.0 using HCl.

Buffer C-T (denaturing wash buffer, 1 liter):

8 M Urea	480.50 g urea (60.06 g/mol)
100 mM NaH_2PO_4	13.80 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
10 mM Tris·Cl	12.10 g Tris·Cl (MW 121.1 g/mol)
0.05% (v/v) Tween 20	5 ml of a 10% (v/v) Tween 20 stock solution

Adjust pH to 6.3 using HCl.

Buffer E-T (denaturing elution buffer, 1 liter):

8 M Urea	480.50 g urea (60.06 g/mol)
100 mM NaH_2PO_4	13.80 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
10 mM Tris·Cl	12.10 g Tris·Cl (MW 121.1 g/mol)
0.05% (v/v) Tween 20	5 ml of a 10% (v/v) Tween 20 stock solution

Adjust pH to 4.5 using HCl.

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Hoffmann-La Roche owns patents and patent applications pertaining to the application of Ni-NTA resin (in certain countries) and to 6xHis-coding vectors and His-labeled proteins. All purification of recombinant proteins by Ni-NTA chromatography for commercial purposes, and the commercial use of proteins so purified, require a license from Hoffmann-La Roche in certain countries.

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