September 2011

QIAsymphony® Ni-NTA Handbook

QIAsymphony Ni-NTA Native Kit

QIAsymphony Ni-NTA Denaturing Kit

For purification of His-tagged recombinant proteins using the QIAsymphony SP

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QIAGEN Sample and Assay Technologies

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Contents

Kit Contents

QIAsymphony Ni-NTA Kits	Native	Denaturing
Catalog no.	**932636**	**932836**
Number of preps	**384 micro preps or 48 medium preps**	
Two reagent boxes containing:		
Reagent Cartridges	2	2
Enzyme Rack	2	2
Piercing Lid	2	2
Benzonase® Nuclease Grade I 99%	6 x 80 µl	6 x 80 µl
Reuse Seal Set*	2	2
Handbook	1	1

* A Reuse Seal Set contains 8 Reuse Seal Strips.

Storage

Ni-NTA Magnetic Agarose Beads are supplied as a 5% (v/v) suspension in 30% ethanol in the bead trough 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.

The enzyme rack containing Lysozyme and Benzonase Nuclease must be stored at –20°C upon arrival and when not in use on the QIAsymphony SP. The enzymes can be stored under these conditions for up to 6 months without any reduction in performance.

The remaining components of the QIAsymphony Ni-NTA Native and Denaturing Kit containing the buffers should be stored at room temperature (15–25°C). The buffers can be stored under these conditions for up to 6 months without any reduction in performance.

Partially used reagent cartridges can be stored for a maximum of two weeks, enabling cost-efficient use of reagents and more flexible sample processing. If a reagent cartridge is partially used, replace the cover of the trough containing the magnetic beads, seal the buffer troughs with the provided Reuse Seal Strips, and close the enzyme tubes with screw caps immediately at the end of the protocol run to avoid evaporation. Store the reagent cartridge at room temperature. Do not store used reagent cartridges at temperatures below 15°C.

To avoid evaporation, the reagent cartridge should be open for a maximum of 15 hours (including run times) at a maximum environmental temperature of 30°C.

Running batches with low sample numbers (<24) will increase both the time that the reagent cartridge is open and the required buffer volumes, potentially reducing the total number of sample preparations possible per cartridge.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN® product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QIAsymphony Ni-NTA Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAsymphony Ni-NTA Native and Denaturing Kit is tested against predetermined specifications to ensure consistent product quality.

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/support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

The following risk and safety phrases apply to components of the QIAsymphonly Ni-NTA Kits:

Ni-NTA Magnetic Agarose Beads

Contains nickel-nitrilotriacetic acid, ethanol: harmful, sensitizer, flammable. Risk and safety phrase:* 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

Product Use Limitations

QIAsymphony Ni-NTA Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

* 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.

Introduction

QIAsymphony Ni-NTA Native and Denaturing Kits are designed for fully automated purification of recombinant 6xHis-tagged proteins from eukaryotic or bacterial cells by magnetic-bead–based immobilized-metal affinity chromatography (IMAC). The kits contain all reagents required for cell lysis and for purification of His-tagged proteins.

The purification principle is based on the remarkable selectivity of unique, patented Ni-NTA (nickel-nitrilotriacetic acid) for proteins with an affinity tag of six consecutive histidine residues — the 6xHis tag. This technology allows purification, detection, and assay of almost any 6xHis-tagged protein from any expression system. The 6xHis tag rarely affects protein structure or function, and for most applications does not need to be removed from the purified protein. In cases where native proteins are desired, such as protein crystallization for structural determination, the TAGZyme™ system offers an extremely efficient solution for exoproteolytic removal of N-terminal affinity tags [1, 2].

Using proven Ni-NTA magnetic-bead technology, QIAsymphony Ni-NTA Kits provide highly pure proteins [3, 4] suitable for direct use in a range of downstream applications, such as structural and functional investigations, activity-based assays, protein–protein, protein–nucleic acid, and protein–ligand interaction assays, and small-animal immunization to produce antibodies.

The QIAsymphony SP performs all steps of the sample preparation procedure, and the procedure can be scaled up or down, allowing purification from varying amounts of starting material. Up to 96 samples are processed in a single run. Results from magnetic-bead–based purification screenings can act as the basis for process development leading to pilot and production scale [2].

Principle and procedure

QIAsymphony Ni-NTA Kits use magnetic agarose beads for purification of recombinant proteins. Magnetic agarose beads combine the proven efficiency of agarose-based protein purification with the convenient handling of magnetic beads. Proteins bind via their tag to the ligand-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 (Figure 1).

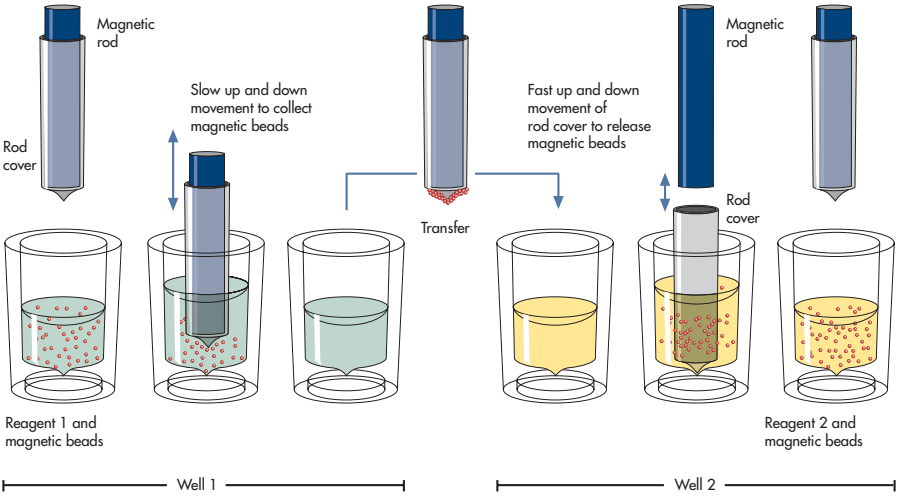

Figure 1. Schematic of the QIAsymphony SP principle. The QIAsymphony SP processes a sample containing magnetic beads as follows: A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic beads. The magnetic rod cover is positioned above another well and the magnetic beads are released. The QIAsymphony SP uses a magnetic head containing an array of 24 magnetic rods, and can therefore process up to 24 samples simultaneously. Steps 1 and 2 are repeated several times during sample processing.

Starting material is *E. coli* or eukaryotic cell pellets in 96-well or 24-well deep-well blocks (see Table 3, page 18).

Purification of 6xHis-tagged proteins using Ni-NTA Magnetic Agarose Beads

Ni-NTA Magnetic Agarose Beads contain magnetic cores and have strongly metal-chelating nitrilotriacetic acid (NTA) groups covalently bound to their surfaces. They are precharged with nickel and ready to use for purifying 6xHis-tagged proteins under native or denaturing conditions. Adjusting the amount of Ni-NTA Magnetic Agarose Beads and therefore the binding capacity allows a choice of the amount of 6xHis-tagged protein captured to suit the particular assay. Protocols are offered for a yield of His-tagged proteins of 20–40 µg (micro-scale) or 250–500 µg (medium-scale) using 20 or 250 µl of bead suspension, respectively. 6xHis-tagged proteins are eluted from Ni-NTA Magnetic Agarose Beads using 500 mM imidazole (native conditions) or by reducing pH (denaturing conditions). Ni-NTA Magnetic Agarose Beads are supplied as a 5% (v/v) suspension with a binding capacity of 1–2 mg protein per ml of suspension for 6xHis-tagged proteins such as Green Fluorescent Protein (GFP).

Purification under native and denaturing conditions

Since the interaction between Ni-NTA and the 6xHis tag of the recombinant protein does not depend on tertiary structure, proteins can be captured and purified under denaturing as well as native conditions. If the protein is found in inclusion bodies, it may be solubilized with strong denaturants, such as urea. Purification under denaturing conditions ensures that all 6xHis-tagged protein in the cell is solubilized and can be captured. In addition, the 6xHis tag is fully exposed under denaturing conditions, which leads to more efficient purification. Ni-NTA Magnetic Agarose Beads specifications are listed in Table 1.

Table 1. Specifications of Ni-NTA Magnetic Agarose Beads

Ligand	Nitrilotriacetic acid (NTA), precharged with nickel
Binding capacity	Up to 2 mg/ml 5% bead suspension for a 6xHis-tagged protein of 30 kDa (~33 nmol/ml) Up to 40 mg/ml settled bed volume for a 6xHis-tagged protein of 30 kDa
Support	Sepharose CL-6B
Bead structure	Cross-linked 6% agarose, magnetic core
Bead size (diameter)	20–70 µm (average 50 µm)
Form	5% bead suspension in 30% ethanol, precharged with nickel
Antimicrobial agent	30% ethanol
Storage	2–8°C
Application	Purification of His-tagged proteins: QIAsymphony micro- to medium-scale Under native conditions using QIAsymphony Ni-NTA Native Kit Under denaturing conditions using QIAsymphony Ni-NTA Denaturing Kit

Figure 2 shows typical results of purification of 6xHis-tagged proteins from Sf9 insect cells under native conditions using the medium-scale protocol and from *E. coli* under denaturing conditions using the micro-scale protocol.

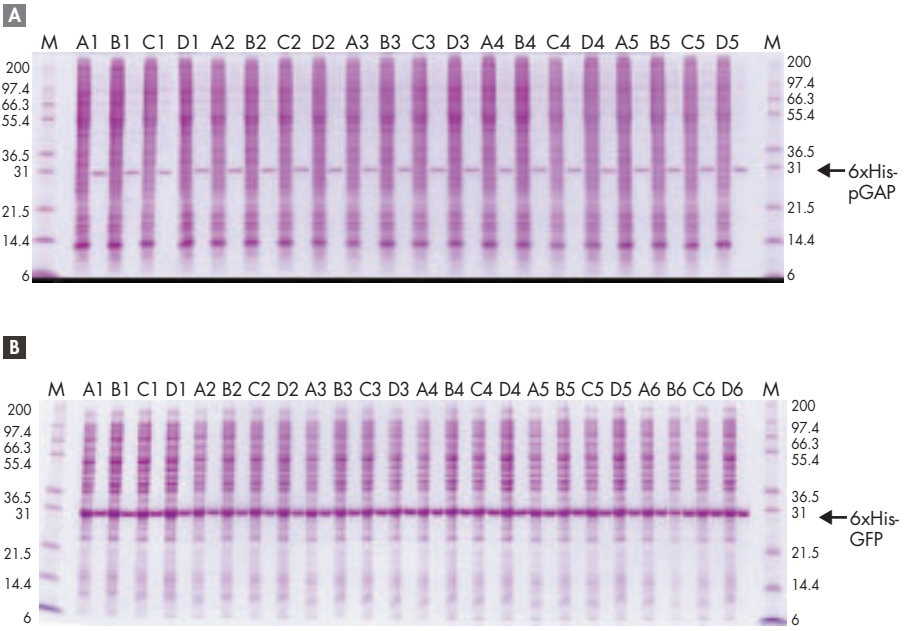

Figure 2. Purification of His-tagged proteins from Sf9 insect and *E. coli* cells using QIAsymphony Ni-NTA kits. A 6xHis-pGAP (pyro-glutamyl aminopeptidase, 275 µg per well) was spiked into Sf9 insect cells (5 ml culture volume, 2.5 x 10^7 cells) and purified by the QIAsymphony Ni-NTA medium-scale protocol under native conditions. On average, >95% of spiked 6xHis-pGAP was recovered with a coefficient of variation (CV) of 8.7%. B 6xHis-GFP (green fluorescent protein) was expressed in *E. coli* M15/[pREP4] using the TAGZyme pQE-2 vector and culture volume aliquots of 1 ml were purified under denaturing conditions using the QIAsymphony Ni-NTA micro-scale protocol. On average of 22 µg protein was purified per well (CV = 5.3%). The well position in the cultivation deep-well block is indicated. **M**: marker (kDa).

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.

- Centrifuge suitable for 24-well or 96-well deep-well blocks (e.g., 4-16K; go to www.qiagen.com for cat. no.)*
- Sample plates (96-well deep-well blocks for 1 ml cultures (e.g., S-Blocks, cat. no. 19585); 24-well deep-well blocks for 5 ml cultures (e.g., 24-Well Blocks RB, cat. no. 19583).* Compatible formats are listed at www.qiagen.com/QIAsymphony/Resources
- Tape cover for cultivation plates (e.g., AirPore Tape Sheets, cat. nos. 120001 or 19571)*
- Elution plates (standard 96-well microtiter plates). Compatible formats are listed at www.qiagen.com/QIAsymphony/Resources
- Cover for elution plates; use either lids provided with 96-well plates or seal plates with adhesive tape recommended for preventing evaporation (e.g., Tape Pads, cat. no. 19570)
- Filter-Tips, 200 µl and 1500 µl (cat. nos. 990332 and 997024)

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

Important Notes

Automated purification on the QIAsymphony SP

The QIAsymphony SP makes automated purification of 6xHis-tagged proteins easy and convenient. Samples, reagents and consumables, and eluates are separated in different drawers. Simply load samples, reagents provided in special cartridges, and preracked consumables in the appropriate drawer before a run. Start the protocol and remove purified protein from the "Eluate" drawer after processing. Refer to the *QIAsymphony SP User Manual* for operating instructions.

"Reagents and Consumables" drawer

Reagent cartridges

Reagents and enzymes for purification of 6xHis-tagged proteins are contained in an innovative reagent cartridge (Figure 3). Each trough of the reagent cartridge contains a particular reagent, such as magnetic beads, lysis buffer, wash buffer, or elution buffer. Enzymes are provided in tubes in the enzyme rack as part of the reagent cartridge. Partially used reagent cartridges can be reclosed with Reuse Seal Strips for later reuse, which avoids generation of waste due to leftover reagents at the end of the purification procedure.

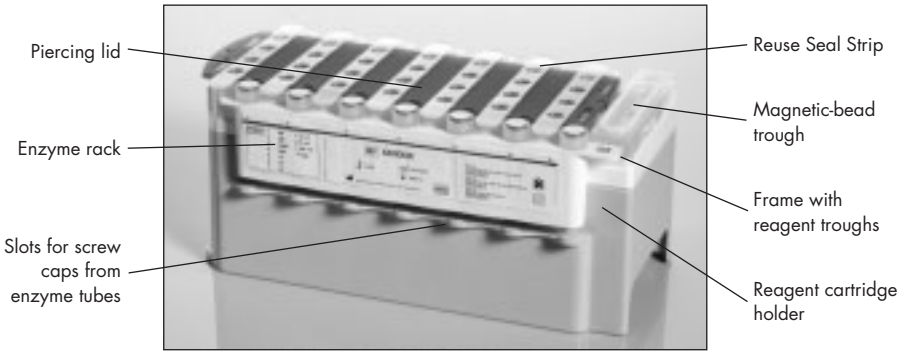

Figure 3. QIAsymphony reagent cartridge. The reagent cartridge contains all reagents required for the protocol run.

Before starting the procedure, ensure that the magnetic beads are settled at the bottom of the bead trough. If beads adhere to the inlet or side walls of the bead trough, resuspend beads by inverting the trough and place back into the reagent cartridge. Place the reagent cartridge into the reagent cartridge holder. If required by the protocol, place the enzyme rack into the reagent cartridge holder. Before using a reagent cartridge for the first time, place the piercing lid on top of the reagent cartridge (Figure 4).

Important: The piercing lid is sharp. Take care when placing it onto the reagent cartridge. Make sure to place the piercing lid onto the reagent cartridge in the correct orientation.

After the magnetic-bead trough cover is removed and the enzyme rack tubes are opened (screw caps can be stored in dedicated slots, see Figure 3), the reagent cartridge is subsequently loaded into the "Reagents and Consumables" drawer.

Figure 4. Easy worktable setup with reagent cartridge.

Partially used reagent cartridges can be stored until needed again, see "Storage", page 4.

Note: Do not store used reagent cartridges from the QIAsymphony Ni-NTA Denaturing Kit at temperatures below 15°C as urea may precipitate.

Replace the lids of the enzyme tubes in the enzyme rack and store the enzyme rack containing Lysozyme/Benzonase Mix or Benzonase Reagent at –20°C.

Loading plasticware

Sample prep cartridges, 8-Rod Covers (both preracked in unit boxes), and disposable filter-tips (200 µl tips provided in blue racks, 1500 µl tips provided in gray racks) are loaded into the "Reagents and Consumables" drawer (see Table 2, page 15).

See Table 2 (page 15) for the consumables required for 6xHis-tagged protein protocols. For plasticware ordering information, see page 31.

Note: Both types of tips have filters to help prevent cross-contamination.

Tip rack slots on the QIAsymphony worktable can be filled with either type of tip rack. The QIAsymphony SP will identify the type of tips loaded during the inventory scan.

Note: Do not refill tip racks before starting another protocol run. The QIAsymphony SP can use partially used tip racks.

"Waste" drawer

Sample prep cartridges and 8-Rod Covers used during a run are re-racked in empty unit boxes in the "Waste" drawer. Make sure that the "Waste" drawer contains sufficient empty unit boxes for plastic waste generated during the protocol run.

Note: Ensure that the covers of the unit boxes are removed before loading the unit boxes into the "Waste" drawer. If you are using 8-Rod Cover boxes for collecting used sample prep cartridges and 8-Rod Covers, ensure that the box spacer has been removed.

A bag for used filter-tips must be attached to the front side of the "Waste" drawer.

Note: The presence of a tip disposal bag is not checked by the system. Make sure that the tip disposal bag is properly attached before starting a protocol run. For more information, see the *QIAsymphony SP User Manual*.

A waste container collects all liquid waste generated during the purification procedure. The "Waste" drawer can only be closed if the waste container is in place. Furthermore, a liquid-level sensor detects the level of liquid in the waste container. The system notifies the user if there is not enough capacity left in the container for liquid waste from the queued batch.

"Eluate" drawer

Load the required elution rack into the "Eluate" drawer. Do not load a 96-well plate onto "Elution slot 4". For purification under native conditions only: if eluates should be cooled, use "Elution slot 1" with the corresponding cooling adapter.

Inventory scan

Before starting a run, the instrument checks that sufficient consumables for the queued batch(es) have been loaded into the corresponding drawers (Table 2, next page).

Table 2. Consumables required for 6xHis-tagged protein protocols

Protocol	Micro native		Medium native		Micro denat.		Medium denat.	
Number of samples	**24**	**96**	**24**	**48**	**24**	**96**	**24**	**48**
Reagent cartridges	1	1	1	2	1	1	1	2
Sample prep cartridges*	18	72	18	36	18	72	18	36
8-Rod Covers†	3	12	3	6	3	12	3	6
1500 µl tips‡	83	311	79	151	99	375	95	183
200 µl tips‡	51	195	51	99	51	195	51	99

* 28 sample prep cartridges/unit box.

† Twelve 8-Rod Covers/unit box.

‡ 32 tips/tip rack; the inventory scan requires additional tips (two 200 µl and eight 1500 µl tips).

Expression of 6xHis-tagged recombinant proteins in *E. coli*

The following procedures may be used as a guideline for cultivation of *E. coli* for overexpression of proteins in 96-well or 24-well blocks.

Note before starting

- For growth in 24-well or 96-well blocks, pilot experiments are recommended to optimize cultivation conditions on-site.

Transformation/selection

1. Transform expression constructs into competent *E. coli* cells and plate onto selective agar medium containing the appropriate antibiotics.
2. Incubate agar plate at 37°C overnight.

Precultures

1. Prepare medium (e.g., LB) containing the appropriate antibiotics and transfer 0.5 ml aliquots into each of the required wells of a 96-well deep-well block under sterile conditions.
2. Pick single colonies using a sterile toothpick, inoculate a single well, allow to stand for 5 min and shake briefly. Alternatively, inoculate precultures from glycerol stocks.
3. Remove the toothpick and cover the block (e.g., using an AirPore Tape sheet).

4. Incubate precultures overnight at 30–37°C, shaking at 150 rpm. The OD_{600} of the precultures after overnight growth should be between 1 and 5.

Main cultures in 96-well deep-well blocks

1. For cultivation in 96-well deep-well blocks (2 ml well volume), add up to 1 ml medium containing the appropriate antibiotic(s) to each well under sterile conditions.
2. Transfer 10–100 µl of overnight preculture from the 96-well block to obtain an OD_{600} of approximately 0.05–0.1.

   To ensure proper adjustment of the OD_{600} after inoculation, we recommend performing pilot experiments to verify the average OD_{600} of precultures under the particular conditions. Adjust the inoculation volume (or shaker speed during precultivation if required) to be able to achieve an OD_{600} of 0.05–0.1.
3. Cover the 96-well block with an AirPore Tape Sheet and incubate cultures at 30–37°C, shaking at 150 rpm, for 2–3 h or until an OD_{600} of 0.8–1.0 is achieved.
4. Induce expression of His-tagged proteins by adding 1 µl of 1 M IPTG (final concentration: 1 mM IPTG) and incubate the cultures at 18–37°C for 4 h or overnight, or until an OD_{600} of ≤ 5 is achieved.

   Avoid using cultures that have an OD_{600} greater than 5. The protocols have been programmed to allow resuspension of pellets derived from culture volumes as indicated in Table 3 (page 18) and of a maximum OD_{600} of 5. Higher biomass per well may lead to incomplete sample processing and may require protocol adjustment for efficient protein recovery.

   To avoid exceeding a final OD_{600} of 5, we recommend monitoring growth in several wells of the 96-well block in pilot experiments under the chosen growth conditions. Reduce the culture volume, shorten the induction phase of the expression cultures, or reduce growth temperature if overnight growth leads to OD_{600} values > 5.
5. Pellet cells by centrifuging 96-well blocks at 1000 x *g* for 10 min in a centrifuge suitable for 24- or 96-well blocks, remove supernatant, and freeze cell pellet at –20°C.

   Note: *E. coli* cells must have been frozen for at least 30 min at –20°C prior to the start of the QIAsymphony procedure to ensure proper lysozyme-mediated cell lysis.

Main cultures in 24-well deep-well blocks

1. For cultivation in 24-well deep-well blocks (10 ml well volume), add 3–5 ml medium containing the appropriate antibiotics to each well under sterile conditions.
2. Transfer 30–500 µl of the overnight preculture from the 96-well block to obtain an OD_{600} of 0.05–0.1.

To ensure proper adjustment of the OD_{600} after inoculation, we recommend performing pilot experiments to verify the average OD_{600} of precultures under the particular conditions. Adjust the inoculation volume (or shaker speed during precultivation if required) to be able to achieve an OD_{600} of 0.05–0.1.

3. Cover the 24-well block with an AirPore Tape sheet and incubate cultures at 30–37°C, shaking at 150 rpm, for 2–3 h or until an OD_{600} of 0.8–1.0 is achieved.
4. Induce expression of His-tagged proteins by adding 5 µl of 1 M IPTG (final concentration: 1 mM IPTG) and incubate the cultures at 18–37°C for 4 h or overnight, or until a OD_{600} of ≤5 is reached.

   Avoid using cultures with an OD_{600} of greater than 5. The protocols have been programmed to allow pellets derived from culture volumes as indicated in Table 3 (page 18) and of a maximum OD_{600} of 5 to be resuspended. Higher biomass per well may lead to incomplete sample processing and may require protocol adjustment for efficient protein recovery.

   To avoid exceeding a final OD_{600} of 5, we recommend monitoring growth in several wells of the 24-well block in pilot experiments under the chosen growth conditions. Reduce the culture volume, shorten the induction phase of the expression cultures, or reduce the growth temperature during induction to 18–30°C if overnight growth leads to OD_{600} values >5.
5. Pellet cells by centrifuging 24-well blocks at 1000 x *g* for 10 min in a centrifuge suitable for 24- or 96-well blocks, remove supernatant, and freeze cell pellet at –20°C.

   Note: *E. coli* cells must have been frozen for at least 30 min at –20°C prior to the start of the QIAsymphony procedure to ensure proper lysozyme-mediated cell lysis.

Expression of His-tagged recombinant proteins in Baculovirus-infected insect cells

Procedures have been described for Baculovirus-infected insect cells that allow expression at high throughput in deep-well blocks [5–7].

Before processing using QIAsymphony Ni-NTA protocols or prior to storage at –20°C, wash insect and mammalian cell pellets with PBS.

Starting material

QIAsymphony protein procedures are designed to purify proteins from cell cultures up to a maximum of 5 ml. The amount of starting material and elution volume ranges used in QIAsymphony protein applications are shown in Table 3 (page 18). For each application, sample and elution volumes can be chosen between micro- and medium-scale to give a yield and concentration of high-purity protein suitable for the subsequent application.

Table 3. Sample and elution volumes used in QIAsymphony protein purification protocols

Application	Protocol name	Culture volume (ml)*	Elution volume (µl)
Native Ni-NTA IMAC	Micro native	0.5–1	75
	Medium native	3–5	200
Denaturing Ni-NTA IMAC	Micro denat.	0.5–1	75
	Medium denat.	3–5	200

* Samples must be provided in 96-well deep-well blocks for micro-scale protocols or 24-well deep-well blocks for medium-scale protocols. To ensure complete cell pellet resuspension the OD_{600} of *E. coli* cultures should not exceed 5. Insect cell number should not exceed a total of 5 x 10^6 cells for a micro- and 2.5 x 10^7 cells for a medium-scale prep.

Sample processing

His-tagged proteins can be purified under native or denaturing conditions using the QIAsymphony SP. Protocols are designed to resuspend and lyse cell pellets and purify His-tagged proteins from crude lysates, i.e., without prior clarification of lysates.

Under native conditions, *E. coli* cells are lysed by lysozyme and lysate viscosity reduction is accomplished by digestion of genomic DNA by Benzonase Nuclease.

Eukaryotic (i.e., insect and mammalian) cells are lysed by the detergent Igepal® CA-630 (formerly Nonidet® P-40) at a concentration of 1.0% (v/v) and the viscosity of the lysate is reduced by the action of Benzonase Nuclease.

Benzonase Nuclease and lysozyme contained in the lysate are reliably removed from the protein prep in the wash steps of the IMAC procedure [2].

Under denaturing conditions, cells are lysed by the action of the chaotrope urea contained in the lysis buffer. Also here, the viscosity of the lysate is reduced by the action of Benzonase Nuclease on genomic DNA, which is removed during the wash steps following the binding reaction. Denaturing buffers are generated when the instrument mixes the denaturant urea with different buffer stock solutions. Increased stringency in the wash buffer and elution of His-tagged proteins from beads by elution buffer are achieved by reduction in the pH of the buffers.

If cleared lysates or cell-free expression reactions (e.g., using EasyXpress® Kits) are used as starting material, the lysis step should be omitted from the protocol. Contact your local QIAGEN Technical Services Department for further details.

If a 96-well block is used as the sample input vessel (micro-scale protocols), elution fractions will be positioned in the same order in the 96-well output plate (i.e., the elution fraction for sample in B2 will be contained in position B2 of the 96-well output plate). If one or two 24-well blocks are used as the sample input vessel (medium-scale protocols), elution fractions will be positioned in the 96-well output plate as shown in Figure 5.

24-well blocks

1	5	9	13	17	21		25	29	33	37	41	45
2	6	10	14	18	22		26	30	34	38	42	46
3	7	11	15	19	23		27	31	35	39	43	47
4	8	12	16	20	24		28	32	36	40	44	48

96-well plate

1	9	17	25	33	41						
2	10	18	26	34	42						
3	11	19	27	35	43						
4	12	20	28	36	44						
5	13	21	29	37	45						
6	14	22	30	38	46						
7	15	23	31	39	47						
8	16	24	32	40	48						

Figure 5. Correspondence between positions of samples and elution fractions when using medium-scale processing.

Protocol: Purification of 6xHis-Tagged Proteins Using the QIAsymphony SP

This protocol is designed for purification of 6xHis-tagged proteins from cell pellets of *E. coli* or eukaryotic cells. Table 4 summarizes the protein purification applications supported by QIAsymphony Ni-NTA protocols.

Table 4. Overview of QIAsymphony Ni-NTA protocols for fully automated purification of His-tagged proteins

Scale	Micro	Medium
Conditions	Native and denaturing	
Starting material	Pellet from *E. coli* or eukaryotic cell culture	
Maximum amount of starting material*	*E. coli*: 1 ml culture at $OD_{600} \leq 5.0$ Eukaryotic: 5×10^6 cells	*E. coli*: 5 ml culture at $OD_{600} \leq 5.0$ Eukaryotic: 2.5×10^7 cells
Sample input vessel†	96-well deep-well block	24-well deep-well block
Maximum number of preps per reagent box	192	24
Elution volume	75 µl	200 µl
Yield per prep	up to 40 µg	up to 500 µg

* If cell density is lower, culture volumes may be increased respectively (e.g., up to 2 ml of a *E. coli* culture of $OD_{600} \leq 2.5$ (*E. coli* micro-scale procedure)).

† Harvest into deep-well blocks by centrifugation if cells are cultivated in different vessels.

Important points before starting

- Ensure that you are familiar with operating the QIAsymphony SP. Refer to the *QIAsymphony SP User Manual* for operating instructions.
- Before beginning the procedure, read "Important Notes" starting on page 12.
- Before using a reagent cartridge for the first time, check that the reagent troughs containing 9.6 M urea (only QIAsymphony Ni-NTA Denaturing Kit) do not contain a white precipitate. If necessary, remove the troughs containing urea from the reagent cartridge and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the troughs in the correct positions. Always store the reagent cartridges at room temperature (15–25°C).
- Try to avoid vigorous shaking of the reagent cartridge otherwise foam may be generated, which can lead to liquid-level detection problems.

- *E. coli* cells must have been frozen for at least 30 minutes at –20°C prior to the start of the QIAsymphony procedure to ensure proper lysozyme-mediated cell lysis. Before beginning the procedure, read "Important Notes" starting on page 12.
- For protein purification under native conditions, use the QIAsymphony Ni-NTA Native Kit; for protein purification under denaturing conditions, use the QIAsymphony Ni-NTA Denaturing Kit.

Things to do before starting

- 60 µl of Benzonase Nuclease Grade I must be added to each lysozyme reagent tube (Native Kit) or Dilution Buffer tube (Denaturing Kit) in the enzyme rack and mixed thoroughly by pipetting up and down.
- Cell pellets should be incubated for 15 minutes at room temperature (15–25°C) before starting the run.
- Before loading the reagent cartridge remove the cover from the trough containing the magnetic beads and open the enzyme tubes containing proteinase K. Make sure that the enzyme has been equilibrated to room temperature. Make sure that the piercing lid is placed on the reagent cartridge or, if using a partially used reagent cartridge, make sure the Reuse Seal Strips have been removed.
- If samples are bar coded, orient sample blocks so that the bar codes face the bar code reader at the left side of the QIAsymphony SP.

Procedure

1. **Ensure that the QIAsymphony SP is switched on.**

   The power switch is located at the bottom, left corner of the QIAsymphony SP.

2. **Ensure the "Waste" drawer is prepared properly, and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.**
3. **Load the required reagent cartridge(s) and consumables (see Table 2, page 15) into the "Reagents and Consumables" drawer, and perform an inventory scan of the "Reagents and Consumables" drawer.**
4. **Load the required elution rack into the "Eluate" drawer.**

   Do not load a 96-well plate onto "Elution slot 4".

   For purification under native conditions only: if eluates should be cooled, use "Elution slot 1" with the corresponding cooling adapter (see "Eluate" drawer, page 14).

5. **Place a 24-well deep-well block(s) (for medium-scale protocol) or 96-well deep-well block (for micro-scale protocol) containing cell pellets into the plate carrier and load into the "Sample" drawer.**

   See ordering information for blocks, page 31. For a full list of compatible vessels, see www.qiagen.com/QIAsymphony/Resources .

   Cell pellets are prepared as described in "Important Notes" (starting on page 12)

6. **Using the touchscreen, enter the required information for each batch of samples to be processed.**

   Enter the following information:

   - Sample information (depending on sample racks used)
   - Protocol ("Assay Control Set") to be run (see Tables 3 and 4 on pages 18 and 20)
   - Elution volume and output position

   After information about the batch has been entered, the status changes from "LOADED" to "QUEUED". As soon as one batch is queued the "Run" button appears.

   The Assay Control Set provides information about internal controls, if applicable.

7. **Press the "Run" button to start processing.**

   All processing steps are fully automated.

   At the end of the protocol run, the status of the batch changes from "RUNNING" to "COMPLETED".

8. **Retrieve the elution rack containing the purified protein from the "Eluate" drawer.**

   The protein is ready to use or can be stored at room temperature (15–25°C, denaturing conditions) or 4°C (native conditions).

   In general, magnetic beads are not carried over into eluates. If carryover does occur, magnetic beads in eluates will not affect most downstream applications. If magnetic beads need to be removed before performing downstream applications, tubes or plates containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean tube.

   If the "Eluate" drawer is closed when a batch is running (e.g., if elution racks that contain eluates are removed), the run will be paused and an inventory scan of the "Eluate" drawer will be performed. A message appears during the scan and must be closed (by pressing the "Close" button) before the run can be restarted.

   Result files are generated for each elution plate.

9. **If the reagent cartridge(s) is only partially used, seal it with the provided Reuse Seal Strips and close the enzyme tubes with screw caps immediately after the end of the protocol run to avoid evaporation.**

10. **Discard used sample tubes, plates, and waste according to your local safety regulations.**

    See page 6 for safety information.

11. **Clean the QIAsymphony SP.**

    Follow the maintenance instructions in the *QIAsymphony SP User Manual.*

12. **Close the workstation drawers, and switch off the QIAsymphony SP.**

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx . The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

		Comments and suggestions
General handling		
	Error message displayed in the touchscreen	If an error message is displayed during a protocol run, refer to "Troubleshooting" in the *QIAsymphony SP User Manual*.
Protein does not bind to the Ni-NTA Magnetic Agarose Beads		
a)	6xHis tag is not present	Sequence ligation junctions to ensure that the reading frame is correct. Check for possible internal translation starts (N-terminal tag) or premature termination sites (C-terminal tag).
b)	6xHis tag is inaccessible	Move tag to the opposite end of the protein. Alternatively, purify under denaturing conditions.
c)	6xHis tag has been degraded	Check that the 6xHis tag is not associated with a portion of the protein that is processed.
d)	Protein not correctly folded	Protein may form inclusion bodies due to incorrect folding. Reduce temperature during induction to 18–30°C.
e)	Protein precipitates during purification	Temperature is too low. Perform purification at room temperature (15–25°C).
Precipitate in reagent trough of opened cartridge		
a)	Buffer evaporation	Excessive evaporation can lead to increased salt concentration in buffers. Discard reagent cartridge. Make sure to seal buffer troughs of partially used reagent cartridges with Reuse Seal Strips when not being used for protein purification.

		Comments and suggestions
b)	Storage of reagent cartridge	Storage of reagent cartridge under 15°C may lead to formation of precipitates. If necessary, remove the troughs containing urea from the reagent cartridge and incubate for 30 min at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the troughs in the correct positions. If the reagent cartridge is already pierced, make sure that thethe trough is reclosed with a Reuse Seal Strip and incubate the complete reagent cartridge for 30 min at 37°C with occasional shaking in a water bath.
Protein elutes with contaminants		
	Contaminants are truncated forms of the tagged protein	Check for possible internal translation starts (C-terminal tag) or premature termination sites (N-terminal tag).
Purification from mammalian cells		
a)	No protein band in SDS-PAGE analysis of the eluate	Expression is too low. Check the expression level by western blotting using an Anti·His Antibody or a protein-specific antibody. Alternatively, perform an immunoassay with Ni-NTA Magnetic Agarose Beads (see the *Ni-NTA Magnetic Agarose Beads Handbook*) or ELISA using Ni-NTA HisSorb Strips (see the *QIA*express® *Detection and Assay Handbook*). If only small amounts of 6xHis-tagged protein are present in the lysate, increase the amount of starting cell material to the maximum allowed (refer to Table 4, page 20).
b)	6xHis tagged protein has been degraded	Check that the 6xHis tag is not removed from the protein during post-translational processing.
c)	6xHis-tagged protein partially elutes in the wash	The binding capacity used is too low to bind all of the 6xHis-tagged protein. Twenty microliters of magnetic bead suspension has a binding capacity of 40 µg 6xHis-tagged protein. If significantly larger amounts of 6xHis-tagged protein are present in the lysate, reduce the amount of starting material accordingly. Alternatively, you may increase the amount of beads; contact QIAGEN Technical Services for more information.

Binding of contaminants

Amount of starting material too low	Match the amount of starting material (6xHis-tagged protein to be purified) to the total binding capacity of the beads by adjusting the amount of the culture volume processed per well (see Table 4, page 20), or switch to the micro-scale protocol if appropriate. Proteins that contain neighboring histidines are not common in bacteria, but do occur in eukaryotic cells. These proteins, as well as endogenous proteins with metal-binding sites, normally bind with lower affinity to the Ni-NTA matrix than 6xHis-tagged proteins. If the binding capacity of the amount of beads used greatly exceeds the amount of 6xHis-tagged protein to be purified, these proteins might bind to the Ni-NTA matrix to a considerably higher extent, and might subsequently be recovered in the eluate.

Purification from insect cells

No protein band in SDS-PAGE analysis of the fractions

a)	No or low expression	Check the expression level by western blotting using an Anti·His Antibody or a protein-specific antibody. Alternatively perform an ELISA using Ni-NTA HisSorb Strips (see *QIAexpress Detection and Assay Handbook*). If low amounts of 6xHis-tagged protein are present in the lysate, increase the amount of starting cell material to the maximum allowed (see Table 4, page 20).
b)	6xHis-tagged protein has been degraded	Check that the 6xHis tag is not removed from the protein during post-translational processing or by endogenous proteases during the purification procedure.

	Comments and suggestions
c) 6xHis-tagged protein partially elutes in the wash buffer or flow-through	The amount of matrix used is too low to bind all of the 6xHis-tagged protein. Twenty microliters of magnetic Ni-NTA Magnetic Agarose Beads has a binding capacity of 40 µg 6xHis-tagged protein. If significantly larger amounts of 6xHis-tagged protein are present in the lysate, reduce the amount of starting material accordingly. Alternatively, you may increase the amount of beads; inquire for assistance for changes in the protocol.
Contaminants bind to resin	
Amount of starting material too low	Match the amount of starting material (6xHis-tagged protein to be purified) to the total binding capacity of the beads by adjusting the amount of the culture volume processed per well (see Table 4, page 20), or switch to the micro-scale protocol if appropriate. Endogenous proteins with metal-binding sites normally bind with lower affinity to the Ni-NTA matrix than 6xHis-tagged proteins. If the binding capacity of the amount of matrix used greatly exceeds the amount of 6xHis-tagged protein to be purified, these proteins will bind to the Ni-NTA matrix, and subsequently will be recovered in the eluate.
General points to consider	
Elution fraction contains protein impurities	
Volume of sample too low	Amount of His-tagged protein present in the sample does not match the binding capacity of the beads. Check amount of starting material (compare to guidelines given in Table 4, page 20, for allowed ranges).
Lower than expected protein yields	
Cell pellets not efficiently resuspended	See Table 4 (page 20) for guidelines for amount of starting material for the given protocol and pages 15–17 for recommendations on how to pellet cells by centrifugation.
Magnetic beads aggregate	
Inappropriate storage temperature	Store Ni-NTA Magnetic Agarose Beads at 2–8°C.

Appendix: Buffers and Reagents

Buffers for purification of 6xHis-tagged proteins from *E. coli* and eukaryotic cells under native conditions using QIAsymphony Ni-NTA Kits:

PBS (1 liter)

50 mM NaH_2PO_4*	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
150 mM NaCl*	8.77 g NaCl (MW 58.44 g/mol)

Adjust pH to 7.2 using NaOH.*

NPI-10-TIg (QIAsymphony Ni-NTA Lysis Buffer, 1 liter; labeled NPI1 in the QIAsymphony Ni-NTA Native Kit)

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)
10 mM imidazole*	0.68 g imidazole (MW 68.08 g/mol)
1.0% (v/v) Igepal CA-630*	10 ml Igepal
0.05% (v/v) Tween® 20*	5 ml of a 10 % (v/v) Tween 20 stock solution
0.02% Sodium azide	2 ml of a 10 % (v/v) NaN_3 stock solution

pH adjusted to 8.0 using NaOH.

Lysis of *E. coli* is accomplished by addition of 1 mg/ml lysozyme and 3 units Benzonase Nuclease per ml of original culture volume.

Lysis of insect and mammalian cells is mediated by the detergent Igepal CA-630 in the buffer, and viscosity of the lysate reduced by action of Benzonase Nuclease.

NPI-20-T (QIAsymphony Ni-NTA Wash Buffer, 1 liter; labeled NPI2 in the QIAsymphony Ni-NTA Native Kit)

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
0.02% Sodium azide	2 ml of a 10 % (v/v) NaN_3 stock solution

pH adjusted to 8.0 using NaOH.

NPI-500-T (QIAsymphony Ni-NTA Elution Buffer, 1 liter; labeled NPI3 in the QIAsymphony Ni-NTA Native Kit)

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)
500 mM imidazole	34.04 g imidazole (MW 68.08 g/mol)
0.05% (v/v) Tween 20	5 ml of a 10% (v/v) Tween 20 stock solution
0.02% Sodium azide	2 ml of a 10 % (v/v) NaN_3 stock solution

pH adjusted to 8.0 using HCl.

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

Urea (component 1 of QIAsymphony denaturing buffers, 1 liter; labeled UA in the QIAsymphony Ni-NTA Denaturing Kit)

9.6 M Urea*	576.58 g urea (60.06 g/mol)

Buffer NTT-7.5 (component 2 of QIAsymphony denaturing lysis buffer, 1 liter; labeled NTT1 in the QIAsymphony Ni-NTA Denaturing Kit)

370 mM NaH_2PO_4*	51.06 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
185 mM Tris·Cl*	22.36 g Tris·Cl (MW 121.1 g/mol)
0.185 % (v/v) Tween 20*	18.5 ml of a 10% (v/v) Tween 20 stock solution
0.02% Sodium azide*	2 ml of a 10 % (v/v) NaN_3 stock solution

pH adjusted to 7.5 using HCl.*

Buffer NTT-5.6 (component 2 of QIAsymphony denaturing wash buffer, 1 liter; labeled NTT2 on the QIAsymphony Ni-NTA Denaturing Kit)

370 mM NaH_2PO_4	51.06 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
185 mM Bis-Tris·Cl*	38.71 g Tris·Cl (MW 209.24 g/mol)
0.185 % (v/v) Tween 20	18.5 ml of a 10% (v/v) Tween 20 stock solution
0.02% Sodium azide	2 ml of a 10 % (v/v) NaN_3 stock solution

pH adjusted to 5.6 using HCl.

Buffer NTT-3.0 (component 2 of QIAsymphony denaturing elution buffer, 1 liter; labeled NTT3 on the QIAsymphony Ni-NTA Denaturing Kit)

370 mM NaH_2PO_4	51.06 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
185 mM Bis-Tris·Cl	38.71 g Tris·Cl (MW 209.24 g/mol)
0.185 % (v/v) Tween 20	18.5 ml of a 10% (v/v) Tween 20 stock solution
0.02% Sodium azide	2 ml of a 10 % (v/v) NaN_3 stock solution

pH adjusted to 3.0 using HCl.

* 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.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

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Ordering Information

Product	Contents	Cat. no.
QIAsymphony Ni-NTA Native Kit	For 384 micro-scale preps or 48 medium scale preps: Includes 2 reagent cartridges and enzyme racks and accessories	932636
QIAsymphony Ni-NTA Denaturing Kit	For 384 micro-scale preps or 48 medium-scale preps: Includes 2 reagent cartridges and enzyme racks and accessories	932836
Related products		
Accessory Trough (10)	For use with the QIAsymphony SP	997012
Reagent Cartridge Holder (2)	For use with the QIAsymphony SP	997008
Sample Carrier, plate, Qsym	Plate carrier for sample input. For use with the QIAsymphony SP	9017660
Tube Insert, 11 mm, sample carrier, Qsym (24)	Primary tube adapter (11 mm) for use with the QIAsymphony tube carrier	9241033
Tube Insert, 13 mm, sample carrier, Qsym (24)	Primary tube adapter (13 mm) for use with the QIAsymphony tube carrier	9241034
Tube Insert, 2 ml, sample carrier, Qsym	Secondary tube adapter (for 2 ml screw cap tubes) for use with the QIAsymphony tube carrier	9241032
Cooling Adapter, tubes, 2 ml, Qsym	Cooling adapter for 2 ml screw cap tubes for use in the QIAsymphony "Eluate" drawer	9018088
Cooling Adapter, EMT, Qsym	Cooling adapter for 2 ml for EMT plates for use in the QIAsymphony "Eluate" drawer	9018086
Cooling Adapter, MTP, RB, Qsym	Cooling adapter for round bottom microtiter plates (MTP) for use in the QIAsymphony "Eluate" drawer	9018085
Cooling Adapter, PCR, Qsym	Cooling adapter for PCR plates for use in the QIAsymphony "Eluate" drawer	9018087

Ordering Information

Product	Contents	Cat. no.
S-Blocks (24)	96-well blocks with 2.2 ml wells, 24 per case	19585
24-Well Blocks RB (24)	24-well blocks with 10 ml round-bottom wells, 24 per case	19583
Adapter, tubes, 2 ml, Qsym	Adapter for 2 ml screw cap tubes for use in the QIAsymphony "Eluate" drawer	9018577
Centrifuge 4K15C (220 V, 50 Hz)	Universal refrigerated laboratory centrifuge with brushless motor (220 V, 50 Hz)	81220
AirPore Tape Sheets (50)	Microporous tape sheets for covering 96-well blocks: 50 sheets per pack	19571
AirPore Tape Sheets (25)	Microporous tape sheets for covering 96-well blocks: 25 sheets per pack	120001
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570
Sample Prep Cartridges, 8-well (336)	8-well Sample Prep Cartridges for use with the QIAsymphony SP	997002
8-Rod Covers (144)	8-Rod Covers for use with the QIAsymphony SP	997004
Filter-Tips, 200 µl (1024)	Sterile, Disposable Filter-Tips, racked; (8 x 128)	990332
Filter-Tips, 1500 µl (1024)	Sterile, Disposable Filter-Tips, racked; (8 x 128)	997024
Tip Disposal Bags (15)	For use with the QIAsymphony	9013395

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Notes

Notes

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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Switzerland ▪ Orders 055-254-22-11 ▪ Fax 055-254-22-13 ▪ Technical 055-254-22-12

UK ▪ Orders 01293-422-911 ▪ Fax 01293-422-922 ▪ Technical 01293-422-999

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