



QuickPick™ IMAC

62301 • metal affinity kit for proteins, 8 preps

62311 • metal affinity kit for proteins, 48 preps

INTRODUCTION

These are the instructions for use for the QuickPick™ IMAC kit. Please read the entire instructions carefully before starting to work with the reagents. The QuickPick IMAC reagents are intended for use with the PickPen® magnetic tools supplied by Bio-Nobile. Also refer to the PickPen® instructions for use. The QuickPick IMAC kit provides a fast and simple means for recombinant histidine-tagged (His-tag) protein purification based on metal affinity using Ni²⁺. PickPen 1-M is recommended when working in microtube format and PickPen 8-M when working in microplate format typically with smaller sample sizes and higher throughput.

SPECIFICATIONS

Recommended vessel format:	1.5 ml microtubes, 96-well plates (U-bottom, minimum volume of 300 µl is recommended)
Yield per preparation:	30 µg ± 3 µg (His-tagged glutathione-S-transferase)
Sample volume:	1-5 ml of cell culture (The amount of culture required depends on the level at which the protein is expressed)
Elution volume:	50 µl
Total protocol time:	5 min

KIT CONTENTS

	62301	62311
IMAC Magnetic Particles	0.8 ml	5.0 ml
IMAC Regeneration Buffer	3.5 ml	20 ml
IMAC ^{2x} Wash Stock Buffer*	10 ml	2 x 30 ml
IMAC Imidazole Buffer, 500 mM	0.8 ml	5.0 ml
IMAC Elution Buffer	0.8 ml	5.0 ml
8-Pack PickPen® tips	1 pack	6 packs

IMAC Magnetic Particles:	IMAC magnetic particles in phosphate buffer (pH 7.0), NaCl, Tween 20, 0.02 % NaN ₃
IMAC Regeneration Buffer:	Aqueous NiSO ₄ solution, Tween 20, 0.02 % NaN ₃
IMAC ^{2x} Wash Stock Buffer*:	Phosphate buffer (pH 7.0), NaCl, Tween 20, 0.04 % NaN ₃
IMAC Imidazole Buffer, 500 mM:	500 mM imidazole in phosphate buffer (pH 7.0), 0.02 % NaN ₃
IMAC Elution Buffer:	20 mM Tris-HCl (pH 8.0), 250 mM NaCl, 300 mM imidazole, 0.05 % Tween 20, 0.02 % NaN ₃

* IMAC ^{2x} Wash Stock Buffer is used for the preparation of IMAC Wash Buffers 1, 2 and 3.

ADDITIONAL MATERIAL REQUIRED BUT NOT SUPPLIED WITH THE KIT

1. PickPen[®] 1-M or PickPen[®] 8-M magnetic tool. See also the PickPen[®] instructions for use.
2. Microtubes or 96-well microplates (U-bottom).
3. Orbital shaker (for microplates)

PRINCIPLE OF THE METHOD

QuickPick IMAC Magnetic Particles are formed from magnetic agarose particles which provide an extensive porous surface area especially suitable for the purification of His-tag proteins from cell lysates. The transfer of magnetic particles with PickPen[®] technology enables rapid purification of His-tag recombinant proteins. The methodology removes the need of using time-consuming column technology.

Immobilized metal ion affinity chromatography (IMAC) was originally introduced as a method for group separation, but today it represents one of the most important tools for the single-step purification of proteins based on polyhistidine tags. The principle of the mechanism is based on the interaction between a metal ion coordinated to a covalently bound chelating ligand and a His-tag protein.

PROCEDURE

PickPen[®] tips

The PickPen[®] tips in the 8-Pack are sterile and ready to use. The tips packed in bulk quantities in plastic bags are not sterile, but can be autoclaved (+121 °C at least 20 min) provided that they are first removed from the bag. The separately available PickPen[®] tip box can also be autoclaved.

Note: Tips should be picked up gently from the pack. Too much pressure may open the pack.

The following notes are important for the procedure:

1. To avoid foaming, only mild pulse vortexing is recommended for all solutions (contain Tween 20).
2. Mix the IMAC Magnetic Particles suspension thoroughly before pipetting into microtubes.

3. The volume of the sample used may be between 300-1000 μl when working with PickPen[®] 1-M, and concentrated samples may be diluted using IMAC Wash Buffer 1 as needed.
4. The volume of IMAC Elution Buffer may be between 20-100 μl depending on the protein concentration required for the downstream application.
5. If parallel reactions are performed simultaneously with PickPen[®] 1-M the next reaction may be started while the first sample is being incubated with magnetic particles in the sample tube. The PickPen[®] tip of the first reaction can be stored in an extra tube or in the PickPen[®] tip 8-Pack during the preparation of the next reaction. Remember to mix the sample tube of the first reaction occasionally.
6. To decrease the unspecific adsorption of contaminating proteins, 400 μl of IMAC Wash Buffer 3 can be used in an optional wash step before the elution step. See section: **"Preparing IMAC Wash Buffers 1, 2 and 3"**.
7. In order to obtain higher yields the sample incubation time can be increased from 2 minutes up to 15 minutes. Typically yields will increase > 30 %.

Sample preparation

It is possible to use the QuickPick IMAC kit for a wide variety of sample preparations, where purification of His-tag engineered proteins is needed. IMAC Wash Buffer 1 is recommended as the sample buffer. To avoid unspecific protein binding in the sample, the concentration of NaCl may be adjusted up to 1 M. Also, imidazole may be added up to a concentration of 5 mM. Add protease inhibitors, protein stabilizers or other additives into Wash buffer 1, if needed.

Sample preparation from bacterial and yeast cells or cultivated tissue cells

The most commonly used methods for disruption of cells are ultrasonication and French press. Bead mills are used for tough-to-disrupt cells like yeast.

Example 1: For one preparation suspend *E. coli* cell pellet (from 1-5 ml fermentation) or cultivated tissue cell pellet ($0.5\text{-}2 \times 10^7$ cells) into 300 μl (150 μl for 8-M) of IMAC Wash Buffer 1. Sonicate the suspension on an ice-bath with 10 x 5 s pulses. Leave a 10 s interval without sonication between each pulse to prevent warming of the suspension. After sonication, centrifuge the suspension for 5 minutes at 10000 x g and use the supernatant as the sample.

Example 2: For one preparation suspend yeast cell pellet (100-300 mg) in 300 μl (150 μl for 8-M) of IMAC Wash Buffer 1. Add glass beads to 1/3 volume of the microtube and mix for 5 minutes in a bead mill. After cell disruption, centrifuge the suspension for 5 minutes at 10000 x g and use the supernatant as a sample.

Do not exceed 150 μl volume for 8-M protocol to avoid liquid spilling from the microplate wells during mixing. Dilute samples do not need to be concentrated; however, the volume limit for microtube format is 1 ml. The amount of sample required depends on the level at which the protein is expressed, which must be determined empirically for each expression experiment. If the protein is not expressed efficiently, cells from a larger culture volume should be used.

Preparing IMAC Wash Buffers 1, 2 and 3

IMAC Wash Buffer 1

Prepare by diluting IMAC ^{2x} Wash Stock Buffer in distilled water as follows. For example, for 8 reactions pipet:

1700 μ l IMAC ^{2x} Wash Stock Buffer
1700 μ l H₂O

Final volume = 3400 μ l

IMAC Wash Buffer 2

Prepare by diluting IMAC ^{2x} Wash Stock Buffer in distilled water and add IMAC Imidazole Buffer, 500 mM to the solution. For example, for 8 reactions pipet:

1700 μ l IMAC ^{2x} Wash Stock Buffer
135 μ l IMAC Imidazole Buffer, 500 mM
1565 μ l H₂O

Final volume = 3400 μ l

The amounts of imidazole and NaCl in the final buffer are 20 mM and 250 mM, respectively.

IMAC Wash Buffer 3 (optional)

To decrease the unspecific adsorption of contaminating proteins 0.2-1.0 M NaCl and 5-40 mM imidazole can be used in an optional wash step. For example, for 8 reactions pipet:

1700 μ l IMAC ^{2x} Wash Stock Buffer
270 μ l IMAC Imidazole Buffer, 500 mM
680 μ l 5 M NaCl (not included in the kit)
750 μ l H₂O

Final volume = 3400 μ l

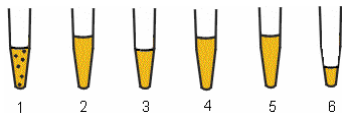
The amounts of imidazole and NaCl in the final buffer are 40 mM and 1000 mM, respectively.

QuickPick™ IMAC kit protocol with PickPen® 1-M

All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved. Make sure that Wash Buffers 1, 2 and 3 (optional) are correctly prepared from the IMAC ^{2x} Wash Stock Buffer before continuing. IMAC Magnetic Particles should be mixed thoroughly just before pipetting. Repeat pipettors should not be used when dispensing magnetic particles.

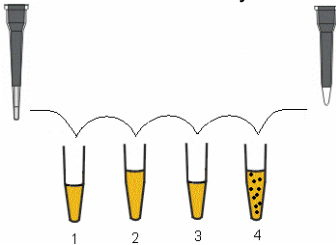
Number tubes from 1 to 6 and pipette liquids into the tubes before starting as follows:

- Tube 1: 100 μ l IMAC Magnetic Particles
- Tube 2: 400 μ l IMAC Regeneration Buffer
- Tube 3: 400 μ l IMAC Wash Buffer 1
- Tube 4: 300 μ l sample (see "Sample preparation")
- Tube 5: 400 μ l IMAC Wash Buffer 2
- Tube 6: 50 μ l IMAC Elution Buffer



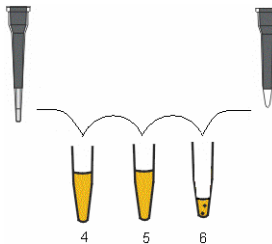
Protocol:

1. Pick up the PickPen[®] tip using the PickPen[®] 1-M. Extend the magnet 2-3 times to check that the tip is firmly in place. Collect the magnetic particles from tube 1 (Magnetic Particles) with PickPen[®] and release them into tube 2 (Regeneration Buffer). If the magnetic particles have formed clumps, they can be broken up by using the PickPen[®] tip. Mix the suspension briefly and gently using the PickPen[®] tip. Note that the magnet has to be withdrawn at this point.
2. Collect the magnetic particles from tube 2 and release them in tube 3 (Wash Buffer 1). Suspend the magnetic particles thoroughly with the PickPen[®] tip and mix briefly and gently. Transfer the magnetic particles from tube 3 into tube 4 (sample). Mix them thoroughly into the solution and incubate at room temperature for at least 2 minutes. Mix the solution occasionally to avoid sedimentation.



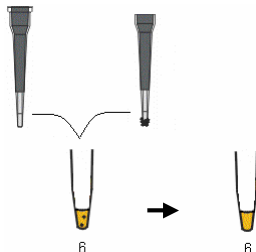
Incubate for at least 2 minutes

3. Collect the magnetic particles from tube 4 and wash them in tube 5 (Wash Buffer 2) by releasing the magnetic particles into the solution and mixing gently for 10 s. An optional wash step may be carried out similarly with Wash Buffer 3 to decrease unspecific protein binding. Collect the magnetic particles from tube 5 and release them into tube 6 (Elution Buffer). Mix the magnetic particles thoroughly in the solution and incubate for at least 1 minute. Mix the solution occasionally to avoid sedimentation.



Incubate for at least 1 minute

4. After incubation collect the magnetic particles from tube 6 and discard them and the tip. The purified His-tagged protein is ready to be used in downstream applications.



Collect the magnetic particles and discard them.

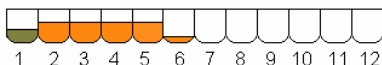
QuickPick™ IMAC kit protocol with PickPen® 8-M

All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved. Make sure that Wash Buffers 1, 2 and 3 (optional) are correctly prepared from the IMAC^{2x} Wash Stock Buffer before continuing. IMAC Magnetic Particles should be mixed thoroughly just before pipetting. Repeat pipettors should not be used when dispensing magnetic particles.

The following instructions are for 8 parallel samples. Samples are lysed in microtubes and transferred into microplates (U-bottom) where the rest of the protocol is carried out.

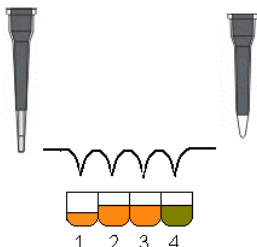
Before starting pipette QuickPick™ IMAC kit reagents into microplate rows 1-6 as follows:

Row 1 (8 wells):	100 µl	IMAC Magnetic Particles
Row 2:	150 µl	IMAC Regeneration Buffer
Row 3:	150 µl	IMAC Wash Buffer 1
Row 4:	150 µl	sample (see “ Sample preparation ”)
Row 5:	150 µl	IMAC Wash Buffer 2
Row 6:	50 µl	IMAC Elution Buffer



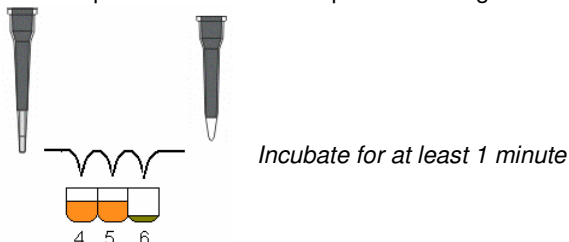
Protocol:

1. Pick up the PickPen® tips using PickPen® 8-M. Extend the magnets 2-3 times to check that the tips are firmly in place. Collect the magnetic particles from row 1 (Magnetic Particles) with PickPen® and release them into row 2 (Regeneration Buffer). If the magnetic particles have formed clumps, they can be broken up by using the PickPen® tips. Mix the suspensions briefly and gently using the PickPen® tips. Note that the magnets have to be withdrawn at this point.
2. Collect the magnetic particles from row 2 with and release them into row 3 (Wash Buffer 1). Suspend the magnetic particles thoroughly with the PickPen® tips and mix briefly and gently. Transfer the magnetic particles from row 3 into row 4 (sample). Mix them thoroughly into the solutions and incubate at room temperature for at least 2 minutes. Mix the microplate using the orbital shaker and make sure that the particles remain in suspension during the mixing.

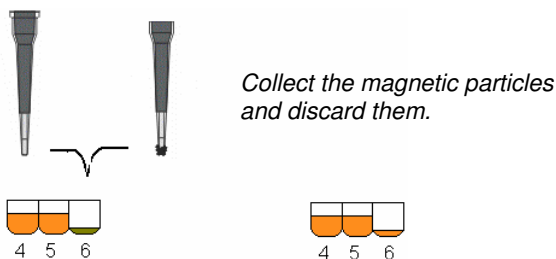


Incubate for at least 2 minutes

3. Collect the magnetic particles from row 4 with and wash them in row 5 (Wash Buffer 2) by releasing the magnetic particles into the solutions and mixing gently for 10 s. An optional wash step may be carried out similarly with Wash Buffer 3 to decrease unspecific protein binding. Collect the magnetic particles from row 5 and release them into row 6 (Elution Buffer). Mix the magnetic particles thoroughly in the solutions and incubate for at least 1 minute at room temperature. Mix the microplate using the orbital shaker. Make sure that the particles remain in suspension during the mixing.



4. After incubation collect the magnetic particles from row 6 and discard them and the tips. The purified His-tagged protein samples are ready to be used in downstream applications.



Processing multiple samples with PickPen[®] 8-M simultaneously, example:

When multiple samples are to be processed an enzymatic treatment for cell lysis is recommended. Freeze the *E. coli* cell pellets (from 1-5 ml culture volume) for at least 1 hour, thaw them and resuspend in 150 μ l of IMAC Wash Buffer 1. Add protease inhibitors, protein stabilizers or other additives, if needed. Lyse the cells by adding lysozyme into the cell suspension into a final concentration of 1 mg/ml. Add DNase up to 5 U/ml of culture volume. Streptomycin sulfate (1 %) can also be used instead of DNase. Incubate for 15 min at room temperature or for 30 min on ice. Centrifuge the lysate for 5 min at 18 000 \times g at room temperature or at +4 °C. Collect the supernatant.

The following instructions are for 16 parallel samples processed on 1 microplate. Samples 1-8 are processed in rows 1-6 and samples 9-16 are processed in rows 7-12. If an optional wash with IMAC Wash Buffer 3 is required only 8 samples can be processed in 1 microplate.

1. Prepare the samples and pipette the liquids into microplate wells as described in “**Sample preparation**” and “**QuickPick IMAC kit protocol with PickPen 8-M**”. Number PickPen[®] 8-Packs; pack nr 1 is for samples 1-8 and pack nr 2 for samples 9-16.

- Pick up the PickPen tips from 8-Pack nr 1 using PickPen® 8-M. Proceed as described in “**QuickPick IMAC kit protocol with PickPen 8-M**” until the magnetic particles from the first series are released into row 4 (samples 1-8).
- Release the PickPen tips into 8-Pack nr 1 and store while handling the next row of samples. Pick up PickPen® tips from 8-Pack nr 2, collect the magnetic particles from row 7 and release them into row 8 (Regeneration Buffer). Proceed as described in the protocol until the magnetic particles from the second series are released into row 10 (samples 9-16).
- When the magnetic particles are in rows 4 and 10 mix the microplate on the orbital shaker for at least 2 minutes at room temperature. Make sure that the particles are in suspension during the incubation step.
- After incubation pick up the PickPen® tips from the 8-Pack nr 1 using PickPen® 8-M and proceed according to the protocol until magnetic particles from both sample series are in rows 6 and 12 (IMAC Elution Buffer). Remember to change the PickPen® tips between the sample series. Mix the microplates on the orbital shaker for at least 1 minute at room temperature. Make sure that the particles are in suspension during the incubation step.
- Pick up the PickPen® tips from 8-Pack nr 1. Collect the magnetic particles from row 6 and discard them and the tips. Proceed similarly with samples 9-16 in row 12 using the tips from pack 2. The eluates containing purified His-tagged protein are ready for downstream applications.

STORAGE AND STABILITY

The QuickPick IMAC kit should be stored at +2°-+8°C. Magnetic particles should not be frozen.

WARNINGS AND LIMITATIONS

The QuickPick IMAC kit is intended for research use only, and not intended for use in human diagnostic or therapeutic procedures.

All solutions contain 0.02-0.04 % sodium azide (NaN₃) as a preservative. When in contact with acid or heavy metal ions, it forms a highly toxic gas. Preservatives such as NaN₃ are toxic if ingested. Do not pipet by mouth. Direct skin contact must be avoided. Appropriate precautions should be taken when handling these solutions. The product contains nickel sulphate (NiSO₄) which is harmful if ingested, inhaled or absorbed through skin contact. Nickel sulphate is a possible carcinogen and teratogen.

DISCLAIMERS AND WARRANTIES

Bio-Nobile warrants that its products shall be free from defects in materials and workmanship and shall meet performance specifications if stored and used in accordance with the instructions for use, for a period up to the expiry date provided on the kit package. This warranty does not cover normal wear and tear or misuse of the product. Bio-Nobile's obligation and the purchaser's exclusive remedy under this warranty is limited to replacement, at Bio-Nobile's expense, of any products defective in manufacture. In no event shall Bio-Nobile be liable for any special, incidental or consequential damages. This warranty statement may be subject to modification in accordance with local laws, regulations and business practices.

PickPen is a registered trademark and QuickPick a trademark of Bio-Nobile Oy.



BIO-NOBILE

Innovations for magnetic bioseparations

Bio-Nobile Oy,
 Tykistökatu 4 B, P.O Box 36, FIN-20521 Turku, Finland
 tel +358 2 410 1100
 www.bio-nobile.com, info@bio-nobile.com